Verigene® Respiratory Pathogens *Flex* Nucleic Acid Test (RP *Flex*)

IVD

REF **20-005-024 (Test Kit) ● 20-012-024 (Amplification Kit)**

RX Only

KEY-CODE **NAN024**

INTENDED USE

The **Verigene® Respiratory Pathogens *Flex* Nucleic Acid Test (RP *Flex*)** is a multiplexed qualitative test intended for the simultaneous detection and identification of multiple viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infection. The test is performed on the automated Verigene System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and microarray hybridization to detect gene sequences of the following organism types and subtypes:

Viruses	Bacteria
Adenovirus	*Bordetella parapertussis/bronchiseptica*
Human Metapneumovirus	*Bordetella holmesii*
Influenza A	*Bordetella pertussis*
Influenza A (subtype H1)	
Influenza A (subtype H3)	
Influenza B	
Parainfluenza 1	
Parainfluenza 2	
Parainfluenza 3	
Parainfluenza 4	
Respiratory Syncytial Virus A	
Respiratory Syncytial Virus B	
Rhinovirus	

Detecting and identifying specific viral and bacterial nucleic acids from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory infection, if used in conjunction with other clinical and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or patient management decisions.

Negative results in the presence of a respiratory illness do not preclude respiratory infection and may be due to infection with pathogens that are not detected by this test or lower respiratory tract infection that is not detected by an NPS specimen. Conversely, positive results do not rule-out infection or co-infection with organisms not detected by **RP *Flex***. The agent(s) detected may not be the definite cause of disease. The use of additional laboratory testing and clinical presentation may be necessary to establish a final diagnosis of respiratory infection.

Clinical evaluation indicates a lower sensitivity specific to **RP *Flex*** for the detection of Rhinovirus. If infection with Rhinovirus is suspected, negative samples should be confirmed using an alternative method.

Performance characteristics for influenza A were established when influenza A/H1 (2009 Pandemic) and A/H3 were the predominant influenza A viruses in circulation. **RP *Flex*** may not detect novel Influenza A strains. If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions used specifically for novel virulent Influenza viruses and sent to appropriate health authorities for testing. Viral culture should not be attempted in these cases unless a biosafety level (BSL) 3+ facility is available to receive and culture specimens.

BACKGROUND AND CLINICAL UTILITY

Respiratory tract infections can be caused by a variety of viral and bacterial organisms. Viruses, notably influenza A, influenza B, and RSV are responsible for the majority of respiratory illnesses and cause significant morbidity and mortality.[1, 2, 3, 4]

Influenza A and B viral infections often result in the respiratory illness commonly referred to as the 'flu'. Flu can lead to serious complications such as pneumonia, bronchitis, sinus infections, encephalitis, and a general worsening of chronic conditions.[5] Flu is highly contagious and, according to the Centers for Disease Control and Prevention (CDC), an average of 20% of the population contract flu each year. Over 200,000 people are hospitalized, and between 3,000 and 49,000 people die of complications, depending on the severity of the flu season. Symptoms include fever, headache, body aches, congestion, fatigue, and general malaise. In the spring of 2009 a novel quadruple-reassortant virus, now known as pandemic A(H1N1) 2009 virus, emerged in North America and quickly spread, becoming a global pandemic by the summer of 2009.[6] The CDC estimates the virus infected between 43 million and 89 million people between April 2009 and April 2010.[7] Importantly, this influenza A subtype was found to be susceptible to the antiviral drug Oseltamivir (brand name Tamiflu), while antiviral resistance varied among other influenza A subtypes.[8,9] Thus, treatment decisions may be impacted by the availability of influenza A subtyping information. The development of acquired immunity to seasonal influenza viruses is limited because influenza viruses mutate in small but important ways from year to year (a process known as antigenic drift). In addition to the risks posed by seasonal influenza viruses, novel influenza viruses have the potential to cause widespread disease and/or disease of unusually high severity because few, if any, people have prior exposure to these viruses. This lack of immunity, as well as additional pathogenic factors that may also increase virulence, results in a greater likelihood of morbidity and mortality among those infected.

Respiratory Syncytial Virus infection is the most common causes of bronchiolitis and pneumonia in infants and children. Each year, 75,000 to 125,000 children in this age group are hospitalized due to RSV infection. Infants who experience RSV infection (especially during the first few months of life) are more prone to wheezing and asthma in later years, although the "cause and effect" relationship remains controversial. RSV is also recognized as a serious contributor to respiratory ailments in the elderly and individuals with weaker immune systems. According to the CDC, flu and RSV occur in temperate climates in community outbreaks that last between 4-6 months persisting through fall, winter, and early spring.[10]

Parainfluenza is the second most commonly identified viral pathogen, especially in young children. Parainfluenza has three prominent serotypes. Infection with human parainfluenza types 1 and 2 typically causes croup. Parainfluenza type 3 causes bronchiolitis and pneumonia.[11] Infection rates for each serotype of parainfluenza were reported in a meta-analysis published in 2001. Type 1 infection is much more common in the United States, leading to between 6,000 and 28,000 hospitalizations among children less than five years of age. Type 2 infection is less common and leads to between 1,800 and 15,600 hospitalizations for children younger

than 5. Type 3 infection is probably the most common type, causing between 8,700 and 52,000 hospitalizations annually among children under five.[12] Parainfluenza type 4 has been poorly studied due to the difficulty of isolation from cell culture. Although epidemiological information is limited, a 2009 study used RT-PCR to analyze nasopharyngeal aspirates from patients admitted to hospitals in Hong Kong that were negative for other respiratory viruses. Human parainfluenza type 4 was detected in 1.2% of patients admitted for respiratory illness who tested negative for other respiratory viruses.[13]

Human Metapneumovirus (hMPV) is the second leading cause of bronchiolitis in young children and is associated with between 5% and 15% of lower respiratory tract infections (LRTI) and approximately 10% of LRTI hospitalizations in young children. Human metapneumovirus is associated with up to 5% of upper respiratory tract infections in young children. Human metapneumovirus has also been detected with lower frequency in adults with respiratory tract infections.[14]

Adenovirus infection can cause a wide range of illnesses, depending upon the specific adenovirus serotype. Respiratory illness is most common and is generally associated with many different serotypes, depending on clinical presentation. Adenovirus infection is dangerous among immunocompromised children. The infection rate in children following bone marrow transplant may be as high as 47%; however, a recent study suggests that the prognosis may be good if the infection is diagnosed and treated early.[15, 16]

Rhinovirus is the most common cause of viral infection in the United States. Rhinovirus infection is generally associated with the common cold but can also cause lower respiratory tract infections. Although rhinovirus infections are generally self-limiting, symptoms can be more serious among immunocompromised patients and patients with underlying respiratory conditions. A 2007 study estimates that the rate of hospitalization associated with rhinovirus infection is 4.8 per 1000 children under five and 25.3 per 1000 children under five with a history of asthma or wheezing.[17] The near-term potential for a new drug for the treatment of rhinovirus further necessitates the need for accurate testing options.[18]

***Bordetella* spp.**, and *Bordetella pertussis,* in particular, cause pertussis, commonly referred to as whooping cough, which is a highly contagious bacterial disease marked by severe coughing fits. In 2012, 48,277 cases of pertussis were reported in the United States.[19] *Bordetella spp.* are small, gram-negative coccobacillus that are obligate aerobe as well as a highly fastidious organism. In addition to *B. pertussis, Bordetella parapertussis* and *Bordetella bronchiseptica* can also cause human infection; however, only *B. pertussis* and occasionally *B. parapertussis* cause pertussis (whooping cough) in humans. Pertussis can cause serious illness in infants (majority of cases in patients < 1 year), children, and adults. The disease usually starts with cold-like symptoms, but after 1 to 2 weeks, severe coughing begins and can continue for weeks. Because pertussis in its early stages appears to be nothing more than the common cold, it is often not suspected or diagnosed until the more severe symptoms appear.[20] The development of a pertussis vaccine in the mid-1980s has minimized death from pertussis in the U.S.[21] However, the incidence of pertussis is on the rise again. Although no one cause has been identified, contributing factors could include better diagnostics, waning immunity, and a decrease in vaccination rates.

Bordetella holmesii is an organism that can cause pertussis-like respiratory tract infections and can be commonly misidentified as *Bordetella pertussis* by conventional diagnostic methods. In an outbreak in Ohio from 2010 – 2011, *Bordetella holmesii* was detected in almost 20% of individuals with pertussis-like illness, which was significantly increased from 1% incidence recorded in earlier outbreaks in the 1990s.[22] The genome of *Bordetella holmesii* does not encode known virulence factors for *Bordetella pertussis,* yet these organisms share a genomic

region that contains the IS*481* insertion element, which is frequently used to diagnose *Bordetella pertussis* with molecular methodologies.[23] This can result in the misidentification of *Bordetella holmesii* as *Bordetella pertussis.* Most United States public health laboratories have historically targeted IS*481*, which is present in 218-238 copies in the *Bordetella pertussis* genome, but only 32-65 copies of the *Bordetella holmesii* genome, for the detection of *Bordetella pertussis*.[23] This has likely led to the under detection and reporting of *Bordetella holmesii* infections.

PRINCIPLES AND PROCEDURES OF VERIGENE RP Flex *AND THE VERIGENE SYSTEM*

RP *Flex* is performed using the Verigene System, which is a bench-top sample-to-result molecular diagnostics workstation consisting of two modules: the Verigene Processor *SP* and the Verigene Reader. The Processor *SP* automates the **RP *Flex*** sample analysis steps including: (i) Specimen Extraction—Magnetic bead-based RNA/DNA extraction from nasopharyngeal swab specimens obtained from symptomatic patients; (ii) Target Amplification--Multiplex RT-PCR- and PCR-based amplification of the extracted nucleic acids to generate target-specific amplicons; (iii) Hybridization—Amplicon hybridization to target specific capture DNA in a microarray format and mediator and gold-nanoparticle probe hybridization to captured amplicons. Silver enhancement of the gold nanoparticle probes bound at the capture sites results in gold-silver aggregates that are imaged optically with high efficiency by the Reader. The Reader also serves as the user interface and central control unit for the Verigene System, storing and tracking information throughout the assay process.

The Processor *SP* utilizes single-use consumables to perform **RP *Flex***, including an Extraction Tray, Amplification Tray and Test Cartridge. A separate Tip Holder Assembly contains two pipette tips that are used to transfer and mix reagents during the assay. The user tests a specimen by loading the single-use consumables into the Processor *SP*, pipetting the prepared specimen into the Extraction Tray, and initiating the protocol on the Verigene Reader by scanning or entering the Test Cartridge ID and specimen information. Following assay completion, the user inserts the Substrate Holder portion of the Test Cartridge into the Reader for optical analysis and generation of **RP *Flex*** test results.

MATERIALS PROVIDED

Verigene RP *Flex* Test Kit (Catalog number **20-005-024**)

- 20 **RP *Flex*** Test Cartridges
  Each Test Cartridge comes preloaded with all required reaction reagents, including wash solutions, oligonucleotide probe solution and signal amplification solutions required to generate a test result. The Test Cartridges are contained within a carrier labeled as: **RP; 20-006-024**

- 20 **RP *Flex*** Extraction Trays (with Tip Holder Assemblies)
  Each Extraction Tray comes preloaded with all required reagents, including lysis/binding buffer, wash solutions, and buffer solutions necessary to extract nucleic acids and generate a test result. The Extraction Trays (with Tip Holder Assemblies) are contained within a carrier labeled as: **RP; 20-009-024**

- 20 Sample Well Caps
  The Caps come packaged in strips of 5 Caps. The Sample Well Caps are contained within a plastic bag labeled as: **40-001-001**

Verigene RP *Flex* Amplification Kit (Catalog number **20-012-024**)

- 20 **RP *Flex*** Amplification Trays
  Each Amplification Tray comes preloaded with all required reagents, including enzymes and buffers necessary to amplify nucleic acids and generate a test result as well as an amplification tube. The Amplification Trays are contained within a carrier labeled as: **RP; 20-011-024**

MATERIALS NEEDED BUT NOT PROVIDED

Instruments and Equipment:

- Verigene Reader; Catalog number 10-0000-02
- Verigene Processor *SP*; Catalog number 10-0000-07
- Barcode Scanner
- 2-8°C Refrigerator
- ≤ -20°C Freezer
- ≤ -70°C Freezer (Optional)
- Micro-pipettors & filtered tips
- Vortex
- Decontamination wipes/spray or comparable sanitizer
- Biological Safety Cabinet (BSC)
- Verigene Extraction Tray Holder; Catalog number 421-00019-01
- Test Cartridge cover opener (Optional)

STORAGE, HANDLING, STABILITY

Table 1: Consumable Storage and Handling

***Verigene* RP *Flex* Test Components**	***Storage Conditions***	***Comments***
Sample Well Caps	2 – 30°C	Do not freeze.
Tip Holder Assemblies		
Extraction Trays		
Test Cartridges	2 – 8°C	
Amplification Trays	≤ - 20°C	Shipped frozen. Upon receipt store frozen. Do not re-freeze after thawing.

VERIGENE DAILY MAINTENANCE

A. Work Area Preparation

Each day of testing and before and after sample preparation, prepare the testing work area by sanitizing the BSC, countertops, vortex mixers, pipettes, and any other equipment used for sample processing with a lint-free decontaminating wipe.

B. Verigene System Cleaning

Prior to the start of testing each day, and after completing a run, perform the following steps for each instrument used for testing.

While wearing fresh gloves, use a lint-free decontaminating wipe to thoroughly wipe the Drawer Assembly of the Verigene Processor *SP* as well as the OPEN/CLOSE button on the front of the Processor *SP*. Do not use the same lint-free decontaminating wipe to clean more than one Processor *SP*.

For the Verigene Reader, use a decontaminating wipe to clean the user Touchscreen, Barcode Scanner and the door of the Analysis Compartment.

Please refer to the Verigene System User's Manual for additional details on routine and daily maintenance.

METHODS

Note: Gloves should be worn whenever handling **RP *Flex*** test components, specimens and while interacting with the Verigene System. Good Laboratory Practice regarding glove changing must be followed when handling test kit components and specimens and while interacting with the Verigene System, in order to prevent contamination of the Verigene System and between samples.

A. Specimen Collection & Storage

Inadequate or inappropriate specimen collection, storage, or transport may yield false-negative results. Due to the importance of specimen quality, training of personnel in the correct manner to perform specimen collection and handling is highly recommended.

1. Use a Nylon or Rayon tipped nasopharyngeal swab (NPS) for specimen collection.
2. Place swab into a vial containing viral transport medium ("VTM": e.g. M4, M4-RT, M5, M6; Universal Transport Media; and Universal Viral Transport Media).
3. Specimens should be stored according to transport media manufacturer's specifications. Specimens used for **RP *Flex*** testing must be tested or stored at 2-8°C within 4 hours of collection, regardless of manufacturer's specifications.
4. Specimens may be stored at 2-8°C for a total of 48 hours from time of collection before testing. (Optional) Once testing is complete, the original NPS specimen may be stored at ≤-70°C for storage up to 30 days. One freeze/thaw is permitted if necessary for repeat testing.

   Note: Repeat tests should be performed from original NPS specimen.

B. Nasopharyngeal Swab Specimen Processing

1. Put on fresh gloves for each NPS specimen.
2. Place NPS specimen in a BSC along with a micropipettor and filtered tips.
3. Wipe down the outside of the specimen vial with a decontaminating wipe.
4. Vortex NPS specimen for 10-15 seconds immediately before loading sample into the Extraction Tray.

C. RP *Flex* Procedure

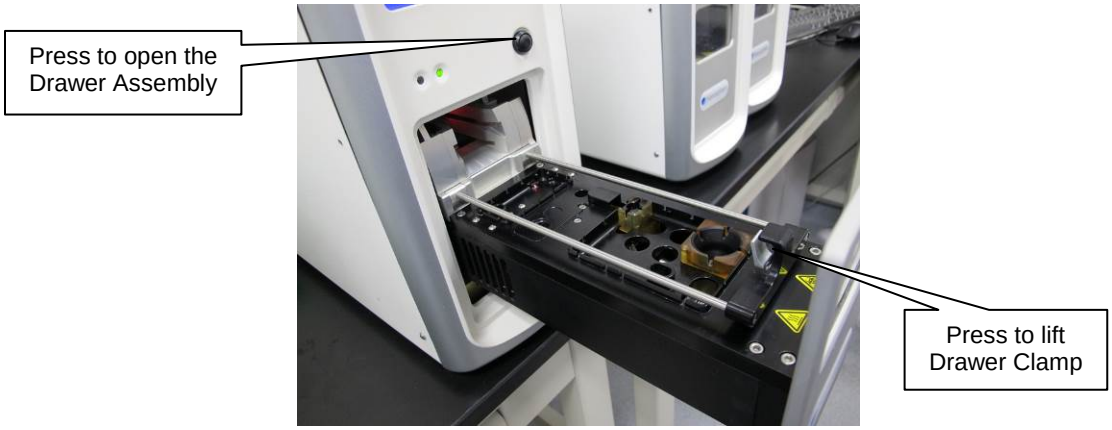
Please refer to the Verigene System User's Manual for additional details on performing rests on the Verigene System.

1. Processor *SP* Set-up
   a) Remove an Extraction Tray, Tip Holder Assembly and Test Cartridge from the refrigerator. Remove the Amplification Tray from the freezer and begin test run within 30 minutes.

      Note: Do not refreeze the Amplification Tray once it has been thawed.

      Note: For Amplification Trays stored at temperatures <-20 °C, thaw the tray at room temperature for at least 10 minutes prior to beginning test run.

   b) Open the Drawer Assembly by pressing the black OPEN/CLOSE button located on the front of the Processor *SP*.
   c) Open the Drawer Clamp by pressing in the silver latch and lifting the Drawer Clamp prior to loading the consumables. The following image shows an empty Processor *SP*.

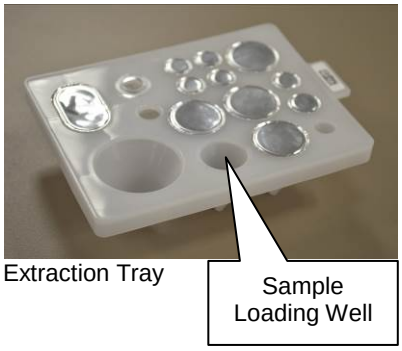


2. Adding Sample to the Sample Loading Well in the Extraction Tray

Note: Samples should be loaded inside a biosafety cabinet (BSC). If a BSC is not used, a dead air box, splash shield, face shield or other personal protective equipment should be used when handling samples, in accordance with the lab's own procedures for handling potentially infectious materials.

a) Remove one Sample Well Cap from the strip and place inside the BSC.
b) Place the Extraction Tray in the Extraction Tray Holder inside the BSC (Refer to image below for Extraction Tray Holder).
c) Gently vortex the sample for 10-15 seconds and pipette 200 µL of the sample into the bottom of the Sample Loading Well in the Extraction Tray (Refer to image below for Sample Loading Well location).

Extraction Tray Holder



Extraction Tray

d) After sample loading, place the Sample Well Cap over the Sample Loading Well. Take precaution to handle only the edges of the Cap and firmly press down until the Cap is fully inserted into the Sample Loading Well.

Sample Well Cap in Packaging

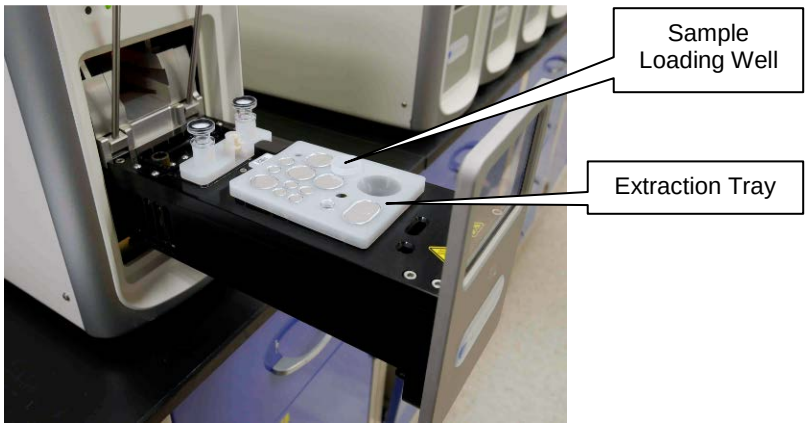
Pressing down on edge of Cap

Extraction Tray with Cap inserted

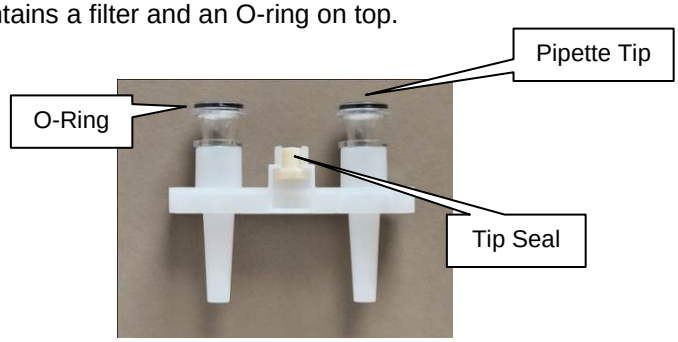
e) Keep the Extraction Tray in the BSC until ready to be inserted into the Extraction Tray Module on the Processor *SP*.

3. Loading the Extraction Tray onto the *Processor SP*

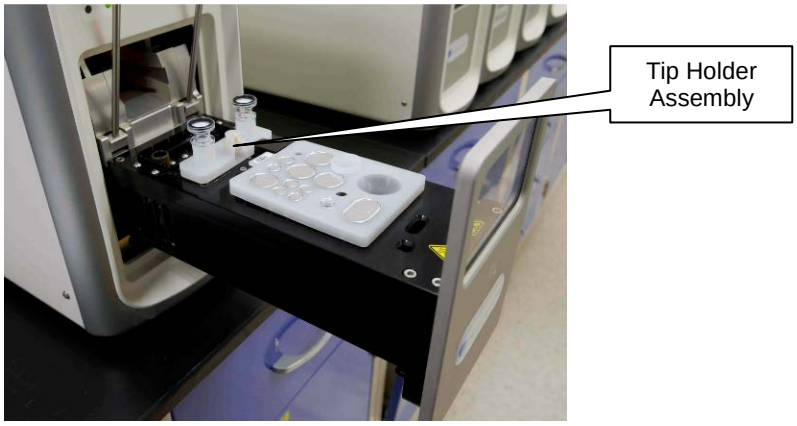
a) The Extraction Tray can only be loaded in one location and orientation in the Drawer Assembly. When the Extraction Tray is loaded correctly, the Sample Loading Well is located at the right hand side of the Drawer Assembly. Place the Extraction Tray in the Drawer Assembly and press down on the corners of the tray to ensure it is level. The image below shows a properly loaded Extraction Tray.



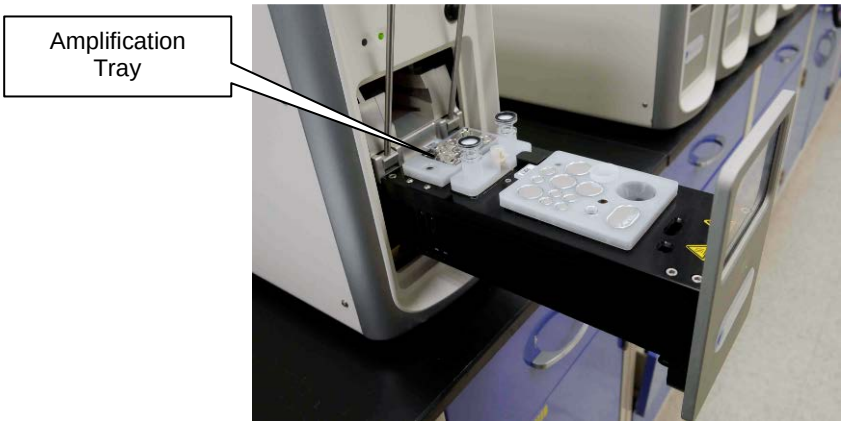
4. Loading the Tip Holder Assembly onto the Processor SP
   a) The Tip Holder Assembly is a plastic holder that contains two Pipette Tips and a rubber Tip Seal. Each Pipette Tip contains a filter and an O-ring on top.



   b) Before using the Tip Holder Assembly, check the top of each Pipette Tip for the O-ring and confirm that the rubber Tip Seal is sitting straight and flush between the tips. If either is missing, replace with a new Tip Holder Assembly.
   c) Insert the Tip Holder Assembly into the Drawer Assembly. The image below shows a properly loaded Tip Assembly. The Tip Holder Assembly can only be loaded in one location and orientation in the Drawer Assembly. For orientation, there are two holes on the deck of the Drawer Assembly that fit each Pipette Tip and the opening to the Tip Seal should face away from Processor *SP*.

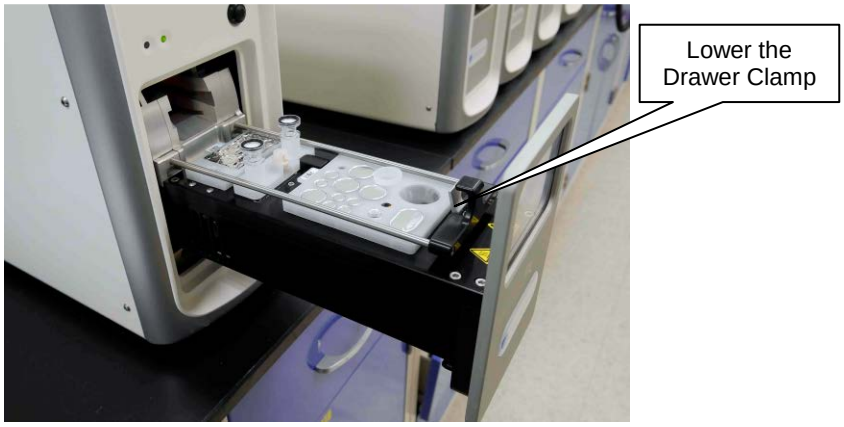


5. Loading the Amplification Tray onto the Processor SP
   a) Remove the cap from the Amplification Tube and save the cap to re-cap the Amplification Tube once processing is complete.
   b) Insert the Amplification Tray into the Drawer Assembly. The Amplification Tray can only be loaded in one location and orientation in the Drawer Assembly. When loaded properly, the tray sits flat. The image below shows a properly loaded Amplification Tray.



   c) Lower and latch the Drawer Clamp over the trays while supporting the Drawer with the opposite hand. The image below shows a closed Drawer Clamp over properly loaded trays and Tip Holder Assembly. The Drawer Clamp will latch onto the Drawer Assembly when closed properly, and the user will be unable to lift the Drawer Clamp without pressing in the silver latch.

      Note: If the Drawer Clamp is not latched properly, the Processor *SP* will display an error message on the Status Display when the user attempts to close the Drawer Assembly.



6. Ordering a Test
   a) All tests must be ordered through the Verigene Reader. No tests can be processed on the Processor *SP* without the user entering the Test Cartridge ID and Sample ID through the Reader.
      i. Log in to the Reader.
      ii. To start a new Session, proceed to the next step (iii). To order a test in a previously created session, select the desired Session from the drop down "SESSION" menu, then proceed to step (v).

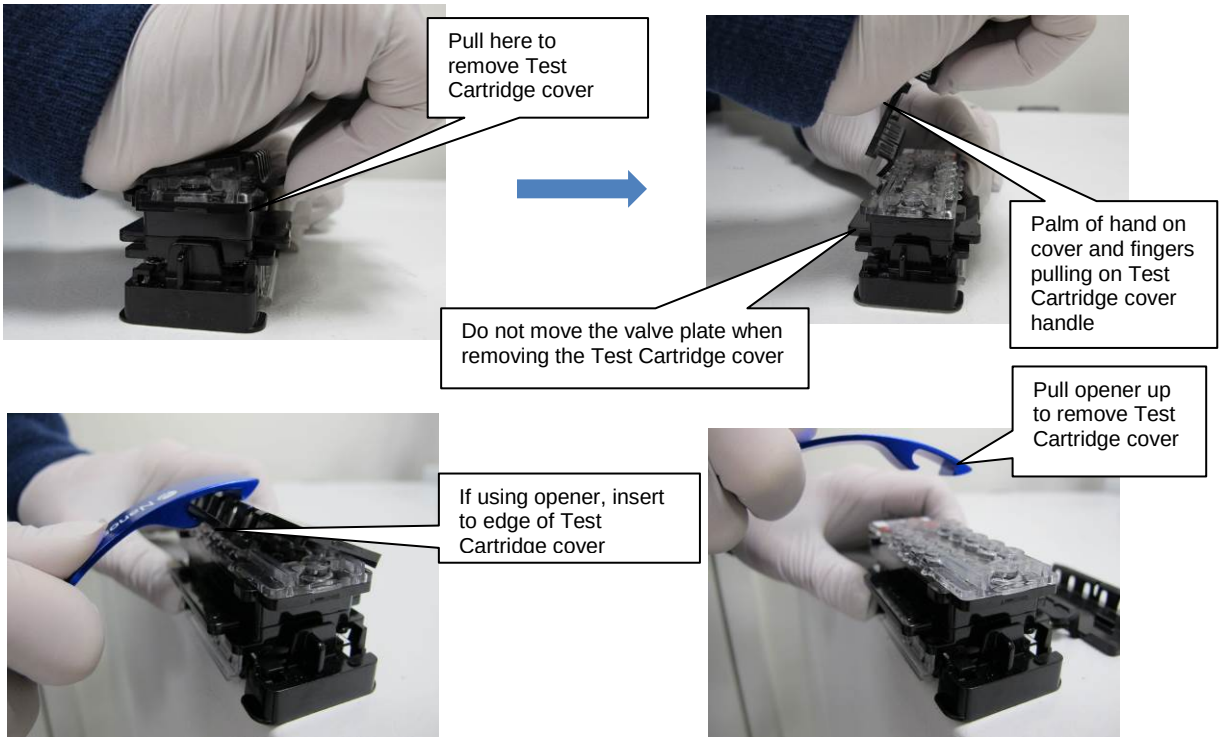
      Note: Up to 60 Test Cartridges can be entered into a single session.

      iii. From the Menu Bar, SESSION tab, select Start New Session where the Session Setup window will appear.
      iv. Touch the "Please Enter" button next to Session ID and enter information by using the data entry keyboard. The Session ID can be any unique identifier in a format defined by the laboratory. The operator ID is automatically entered as the currently logged in user.
      v. Touch the Processing option on the Navigation Bar at the bottom of the screen.
         a. Enter the Test Cartridge ID by scanning the barcode using the Barcode Scanner attached to the Reader. The user may manually enter in the Test Cartridge ID by selecting MENU and 'Enter Barcode' and then keying in the Test Cartridge ID number with the Reader's keyboard.
   b) (*optional*) Scan the Test Cartridge cover's 2D barcode using a gun-style Barcode Scanner to display the Test Cartridge's Reference Number, Expiration Date, and Lot Number on reports.

      Note: The wand-style Barcode Scanner will not read 2D barcodes.

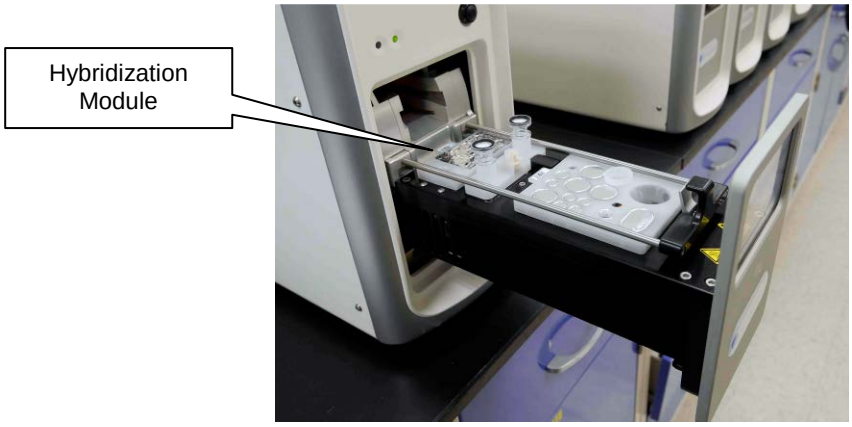
7. Loading a Test Cartridge
   a) Hold the Test Cartridge by the handle with one hand, using the other hand apply pressure with the palm of the hand and remove the Test Cartridge cover by bending the cover away and over the Reagent Pack edge. Ensure that the valve plate is not moved during cover removal (see illustration below).

      Note: Do not remove the Test Cartridge cover until immediately prior to inserting the Test Cartridge into the Processor *SP*.

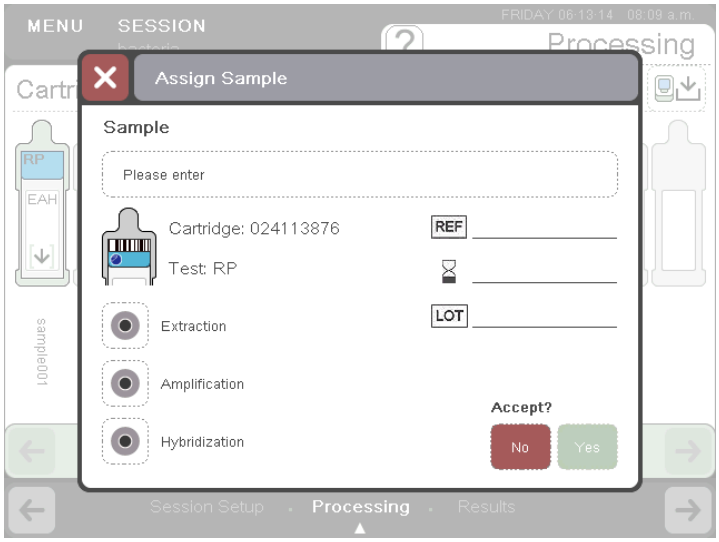

Pull here to remove Test Cartridge cover
Palm of hand on cover and fingers pulling on Test Cartridge cover handle
Do not move the valve plate when removing the Test Cartridge cover
Pull opener up to remove Test Cartridge cover
If using opener, insert to edge of Test Cartridge cover

b) Insert the Test Cartridge into the Hybridization Module of the Processor *SP* until it reaches a stopping point. The image below shows the user loading a Test Cartridge into the Processor *SP*.

Note: If the Test Cartridge is not inserted properly, a message will appear on the Processor *SP* Status Display when the user attempts to close the Drawer Assembly.



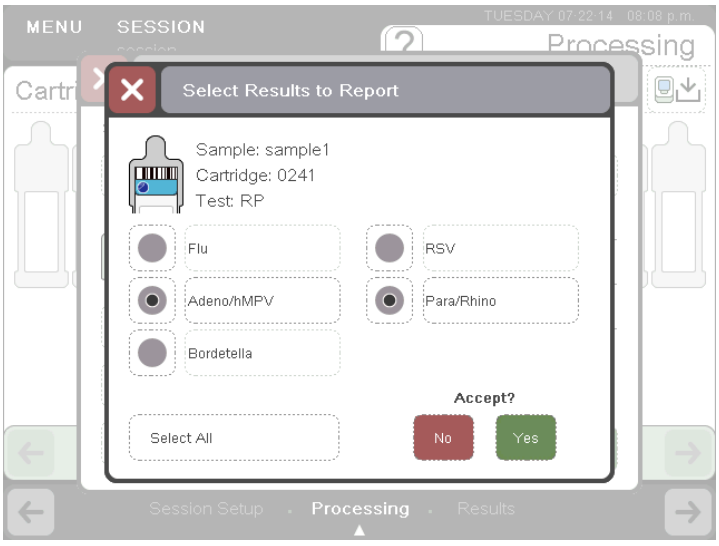
c) On the Reader, enter the sample ID by scanning the barcode or manually enter the sample ID using the data entry keyboard. Press Yes to confirm the sample ID if manually entering. Ensure the Hybridization, Amplification and Extraction options are selected (see image below).



d) In the subsequent dialogue box on the Reader, select or de-select the target groupings to activate or deactivate results reporting for those targets. Target groupings include:

i. "Flu": Influenza A, Influenza A (subtype H1), Influenza A (subtype H3), Influenza B
ii. "RSV": RSV A, RSV B
iii. "Adeno/hMPV": Adenovirus, Human Metapneumovirus
iv. "Para/Rhino": Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, Rhinovirus
v. "Bordetella": *Bordetella parapertussis/bronchiseptica*, *Bordetella holmesii*, *Bordetella pertussis*

Note: Within each target grouping, you can select or de-select targets for reporting. Choose **Select All** to report all **RP *Flex*** targets.



e) Press "Yes" to accept target grouping selections.

Note: The Reader will automatically default to the selected targets for the next test run.

8. Close the Drawer Assembly by pressing the OPEN/CLOSE button on the Processor *SP*. The Processor *SP* will automatically verify that each consumable is properly loaded and begin sample processing.

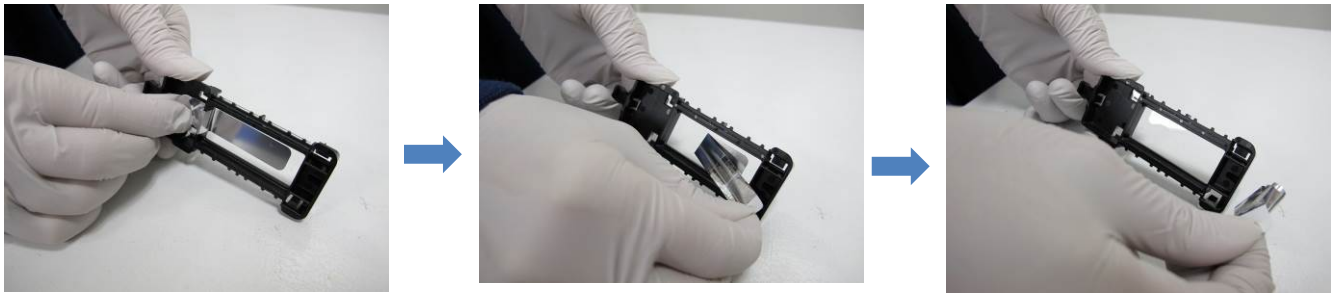
9. Confirm countdown has started on the Processor *SP* Status Display before leaving the area.

10. In order to set up additional tests on other Processor *SP* instruments follow the same procedure. To avoid contamination and sample mix-ups, set up one test at a time.

11. Upon completion of processing
    a) The Reader will generate a ring to notify the user when processing is complete and the Status Display on the Processor *SP* will flash a message indicating "Procedure Done. Ready to Open Drawer."
    b) Open the Drawer Assembly by pressing the OPEN/CLOSE button.
    c) Cap the Amplification Tube for disposal.
    d) Remove the Test Cartridge upon completion or within 12 hours of test completion and immediately orient to its side.
    e) While keeping the Test Cartridge on its side, separate the Reagent Pack (see illustration below).

12. Analyzing results
    a) Remove the protective tape from the back of the Substrate in the Substrate Holder (see illustration below).

    Note: Use precaution not to touch the surface of the Substrate.

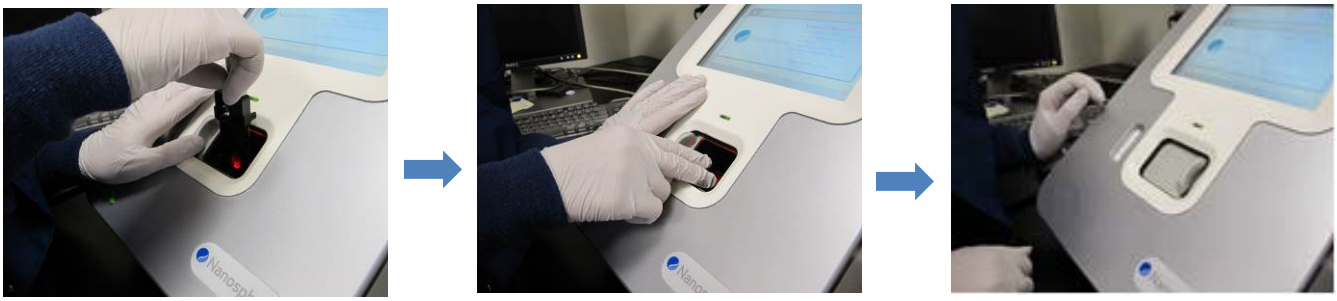


b) Use the Barcode Scanner to scan the barcode on the Substrate. When the barcode is accepted, a prompt to load the Substrate Holder into the Reader will be displayed.

Note: Scanning the barcode ensures the result is associated with the correct sample. When the load Substrate Holder prompt occurs, it will only display for 20 seconds. Analysis will only start if the Substrate Holder is loaded during the animated prompt.

c) Immediately insert the Substrate Holder into the Reader.

Note: To properly insert the Substrate Holder into the Reader, hold the Substrate Holder by the handle with the barcode facing away from you. Next, insert the Substrate Holder into the Analysis Compartment. The compartment is designed to hold the Substrate in the correct position; do not force the Substrate Holder into the Analysis Compartment. Insert the Substrate into the compartment as far as it will go comfortably. There should be an audible "click" sound when the Substrate Holder is inserted properly. Close the door of the Analysis Compartment.



d) Analysis will automatically begin. A small camera icon will appear on the Reader to indicate that analysis has begun.
e) Once the analysis is completed by the Reader, the camera icon will be replaced with an upward facing arrow and the Reader rings.

Note: Confirm that a result other than "No Call - NO GRID" has been generated by touching the substrate icon for the test. A Substrate producing a "No Call - NO GRID" result should be reanalyzed (Refer to **Table 3** in the ***INTERPRETATION OF RESULTS* section**).

f) Once the scan is complete, dispose of the used Substrate Holder and the used Reagent Pack according to applicable regulations.
g) To access the remaining used consumables, raise the Drawer Clamp; remove and dispose of the used Extraction and Amplification Trays, the capped Amplification Tube and the Tip Holder Assembly according to applicable regulations.

13. Printing results
    a) Touch the substrate icon in the session's Processing screen. A window displaying the results will open; touch the "Print" option on this screen to print a Detail Report.
    b) A Summary Report is available by moving to the Results screen of the Session on the bottom Navigation Bar; go to MENU then select "Print Summary." The Summary Report will provide the results for all samples processed within the current Session.

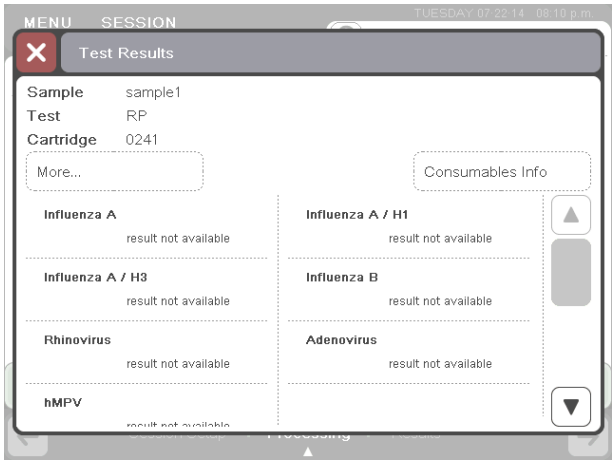
c) Detail Reports can also be viewed and printed from the Results window. First, select the desired Test from the list, go to MENU and then touch "Print Detail."

Note: Targets not selected for reporting will be described as "results not available" in the Summary Report.

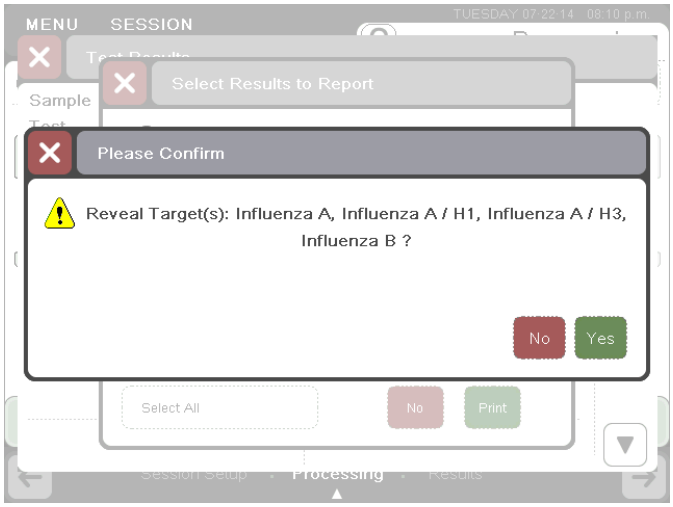
14. At any point following the completion of an **RP *Flex*** test, users may refer back to a previously completed **RP *Flex*** test and reveal testing results for targets not initially selected for reporting by the Reader.

Note: This option is only available for valid Verigene **RP *Flex*** tests. To do this:

a) On the Reader, touch the **SESSION** tab.
b) Select "Fast Results."
c) After the pop-up window appears, touch the "Please Enter" button.
d) Scan the sample barcode / sample ID for the sample of interest or manually enter the sample ID.
e) All results attached to the entered sample ID will be displayed. If multiple tests have been performed on the same sample ID, select the test of interest from the list. If only one test has been performed on the entered sample ID, results will automatically be displayed.
f) Touch the "More…" button.



g) Any targets not previously selected for result reporting are available to reveal testing results. Select the additional target grouping(s) or target(s) that you would like to reveal. Within each target grouping, select or de-select targets to reveal results.
h) Confirm the targets for which you wish to reveal results by selecting "Yes."



15. Print results for the newly revealed target test results.
    a) Upon confirmation of the newly selected targets, touch the “Print” button to print a Detail Report.
    b) A Summary Report is available by moving to the Results screen of the Session on the bottom Navigation Bar; go to MENU then select “Print Summary.” The Summary Report will provide results of all samples processed within the current session.
    c) Detail Reports can also be viewed and printed from the Results window. First, select the desired Test from the list, go to MENU and then touch “Print Detail.”

    Note: Targets not selected for reporting will be described as “results not available” in the Summary Report.

INTERPRETATION OF RESULTS

RP *Flex* provides a qualitative result for the presence (Detected) or absence (Not Detected) of the **RP *Flex*** target genes. The image analysis of the Substrate provides light signal intensities from the target-specific capture spots as well as the internal processing controls, negative control, background, and imaging control spots. The mean signal intensity of a target is compared to the assay's signal detection threshold to make a determination. **Table 2** lists the possible test results generated by **RP *Flex*** representing identification of viral and bacterial nucleic acid sequences/targets; their presence is verified before a valid result is provided as described below.

Table 2: Calls for Valid Tests

Test Result Reported as "Detected"	*Target Genes*
Viral Targets	
Adenovirus	Hexon
hMPV	Polymerase/large protein (L) for species A
	Nucleoprotein (N) for species B
Influenza A*	Matrix protein (M)
Influenza A/H1**	Hemagglutinin (HA)
Influenza A/H3**	Hemagglutinin (HA)
Influenza B	Non-structural protein (NS)
Parainfluenza 1	Fusion protein (F)
Parainfluenza 2	Polymerase/large protein (L)
Parainfluenza 3	Nucleoprotein (NP)
Parainfluenza 4	Phosphoprotein (P)
RSV A	Polymerase/large protein (L)
RSV B	Fusion protein (F)
Rhinovirus	5'-UTR
Bordetella Targets	
*Bordetella parapertussis/bronchiseptica****	*gidA*
B. holmesii	*fumC*
B. pertussis	Toxin promoter region
Test Result Reported as "Not Detected"	
All Analytes "Not Detected"	-

* Detection of influenza A without an influenza A/H1 or influenza A/H3 subtype may occur at low titer of the virus in the specimen or may indicate a false positive due to contamination. The result could also indicate a novel influenza A strain. In these cases the sample should be retested. If an Influenza A detected result is obtained without detection of an Influenza A/H1 or A/H3 subtype upon retesting, contact local or state public health authorities for confirmatory testing.

** Detection of Influenza A/H1 or Influenza A/H3 subtypes without an Influenza A "Detected" result may occur at low titer of the virus in the specimen or may indicate a false positive due to contamination. The result could also indicate potential genetic mutations in the Matrix protein gene among circulating seasonal Influenza A viruses. In these cases, the sample should be retested. If an Influenza A/H1 or A/H3 subtype detected result is obtained again without detection of Influenza A upon repeat testing, further investigations may be warranted.

*** Since the RP *Flex Bordetella parapertussis/bronchiseptica* probes also detect *Bordetella pertussis*, if a *Bordetella pertussis* "Detected" result is obtained, the results for *Bordetella parapertussis/bronchiseptica* are not to be considered as they do not indicate the presence or absence of *Bordetella parapertussis* / *Bordetella bronchiseptica*. The result of *Bordetella parapertussis/bronchiseptica* is reported as "N/A" upon a "Detected" result for *Bordetella pertussis*.

Calls related to an invalid **RP *Flex*** test are listed in **Table** 3 below, together with the appropriate recourse which should be taken by the user.

Table 3: RP *Flex* Invalid Calls and Recourse

Call	*Reason*	*Recourse*
No Call – NO GRID	Reader unable to image Substrate	Ensure Substrate is seated properly in the Substrate Holder. Repeat image analysis by selecting 'Menu' and 'Enter Barcode' and then scanning the Substrate Holder barcode. If the No-Call persists, repeat RP *Flex*
No Call – INT CTL 1	Internal Control 1 not detected, indicating a target hybridization issue.	Repeat RP *Flex*
No Call – INT CTL 2	Internal Control 2 not detected, indicating lysis, extraction, amplification issue, or target hybridization issue.	
No Call – INT CTL	INT CTL 1 and INT CTL 2 not detected, indicating lysis, extraction, amplification, or target hybridization issue.	
No Call – VARIATION	Reader unable to obtain result because of high variability in the target-specific signals	
No Call – BKGD		
No Call – NEG CTL		
Processing Error	Pre-Analysis Error--Internal checks within the Processor *SP* detected an unexpected event.	Power cycle the Processor *SP* and repeat RP *Flex*

QUALITY CONTROL

Quality control, as a component of an overall quality assurance program, consists of tests and procedures for monitoring and evaluating the analytical performance of a measurement system to ensure the reliability of patient test results.

Verigene System

The Verigene System uses a series of automated on-line quality measurements to monitor instrument functionality, software performance, fluidics, test conditions, reagent integrity, and procedural steps each time a test is performed. A series of automated on-line procedural checks guide the user through the testing process each time a test is performed. The **RP *Flex*** test barcode and sample information are linked upon entry into the Reader to help ensure accurate reporting of results.

Assay Controls

Verigene RP *Flex* is a 'sample-to-result' detection system wherein nucleic acids are isolated from respiratory specimen and specific detection is performed on an oligonucleotide array housed within the Test Cartridge. To prevent reagent dispensing errors, all reagents are prepackaged in single-use consumables. Several layers of controls built into **RP *Flex*** ensure that failures at any step within **RP *Flex*** are identified during the procedure or in the end-point image analysis of the Substrate.

Internal Processing Controls

An artificial DNA construct serves as a target hybridization control and is referred to as the Internal Processing Control 1 (INT CTL 1). If the INT CTL1 is not valid, a 'No Call – INT CTL 1' result will be obtained and the test should be repeated.

The bacteriophage MS2 serves as a specimen extraction, amplification & hybridization control and is referred to as the Internal Processing Control 2 (INT CTL 2). This control is automatically added by the Processor *SP* to each specimen prior to the extraction step. If the INT CTL 2 is not valid a 'No Call – INT CTL 2' result will be obtained and the test should be repeated. The following exception exists: INT CTL 1 detection alone is sufficient for a valid call if any of the viral or bacterial targets are also detected; there is no requirement for INT CTL 2 to also be detected. **Table 4** summarizes the two internal processing controls.

If both INT CTL 1 and INT CTL 2 are 'Not Detected', a 'No Call – INT CTL' result will be obtained.

Additional positive and negative controls are immobilized on the Substrate. These are used to determine that hybridization was performed correctly. **RP *Flex*** algorithm requires that these controls be valid before decisions regarding the presence or absence of any other target on the panel can be determined. If these controls are not valid a No Call result will be obtained and the test should be repeated.

Table 4: Internal Processing Controls

Control	*Description*	*Function*
Internal Processing Control (INT CTL 1)	Artificial DNA construct with detection oligonucleotides.	Controls for target hybridization-related issues.
Internal Processing Control (INT CTL 2)	Intact MS2 Phage along with primers and detection oligonucleotides. Added to each test specimen.	Controls for lysis, extraction, target amplification & hybridization.

External Controls

Good laboratory practice recommends running external positive and negative controls regularly. For example, viral transport medium may be used as the external Negative Control, and previously characterized positive samples or negative sample spiked with well characterized target organisms may be used as external Positive Controls. Regardless of the choice of quality control materials, external controls should be used in accordance with local, state, federal accrediting organizations, as applicable.

TROUBLESHOOTING

Refer to the Troubleshooting section of the Verigene System User's Manual.

LIMITATIONS

- Performance characteristics of this product were determined with nasopharyngeal swabs (NPS). Other specimen types have not been validated.
- A trained health care professional should interpret assay results together with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- Viral and/or bacterial nucleic acid may persist *in vivo*, independent of viability. Detection of analyte target(s) does not imply that the corresponding viruses and/or bacteria are infectious, or are the sole causative agents for clinical symptoms.
- The detection of viral and/or bacterial nucleic acid is dependent on proper specimen collection, handling, transport, storage, and preparation, including extraction. Failure to observe proper procedures in any of these steps could lead to incorrect results.
- There is a risk of false negative results due to sequence variants in the viral and/or bacterial targets of the assay, procedural errors, amplification inhibitors in the specimen, or inadequate viral or bacterial concentration for amplification.
- A specimen yielding a negative result may contain respiratory viruses and/or bacteria other than those included in this assay.
- There is a risk of false positive results due to cross-contamination by target viruses and/or bacteria, their nucleic acids or amplified product, or from non-specific signals in the assay. Attention should be given to the handling of consumables under the Warnings and Precautions section to help minimize this risk.
- This assay is a qualitative test and does not provide a quantitative assessment of the concentration of the detected organism.
- The performance of this assay has not been evaluated for patients without signs and symptoms of respiratory infection. Negative results can occur when the cause on an infection is with an organism not on the panel or respiratory tract infections that cannot be detected by a nasopharyngeal swab.
- This assay has not been evaluated for monitoring treatment of Influenza A and/or RSV.
- This assay has not been evaluated for the screening of blood or blood product for the presence of Influenza.
- The performance of this assay has not been established in immunocompromised individuals.
- The effect of interfering substances has only been evaluated for those listed within this document. Interference by substances other than those described can lead to erroneous results.
- Organisms on the panel may have different prevalence depending on the time of year a specimen is tested. Positive and negative predictive values are highly dependent on prevalence. When prevalence is high, false negative results are more likely to occur. When prevalence is low, false positive results are more likely to occur.
- Due to the genetic similarity between human rhinovirus and enterovirus, some strains of enterovirus may be detected as rhinovirus. Cross-reactivity with Human poliovirus 2, Human poliovirus 3, Enterovirus D68, and Coxsackievirus A24 was demonstrated through empirical testing.
- Due to the genetic similarity across the human adenoviruses, this assay is expected to be inclusive to all species. Inclusivity with adenovirus G was based on *in silico* analysis only.
- Influenza A subtyping is based solely on the hemagglutinin gene. No subtyping is performed based on the neuraminidase gene.
- Recent vaccination with the intranasal Influenza vaccine may produce false positive results for influenza A and/or influenza B.
- The *Bordetella parapertussis/bronchiseptica* test is designed to detect *Bordetella parapertussis* and *Bordetella bronchiseptica*. The Verigene **RP *Flex*** probes for the *Bordetella parapertussis/bronchiseptica*

gene target also detect *Bordetella pertussis*. Thus, if a *Bordetella pertussis* "Detected" result is obtained, no determination regarding the presence of *Bordetella parapertussis* or *Bordetella bronchiseptica* can be made as the results for *Bordetella parapertussis/bronchiseptica* are not considered or provided.

- *Due to the small number of positive specimens* collected during the prospective study, performance characteristics of the following targets were established using mainly selected retrospective and contrived samples: influenza A/H3**,** RSV A, parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 3, parainfluenza virus 4, *Bordetella pertussis*, *Bordetella holmesii*, and *Bordetella parapertussis/bronchiseptica*.
- Cross-reactivity with organisms not tested in the Analytical Specificity section may lead to erroneous results.

WARNINGS AND PRECAUTIONS – GENERAL

- **RP *Flex*** is for *in vitro* diagnostic use.
- ***Caution: Federal law restricts this device to sale by or on the order of a physician or to a clinical laboratory***.
- Never use any Tips, Trays, Tubes, or Test Cartridges which have been broken, cracked, punctured, previously used or visibly damaged; using damaged material may lead to No Calls or false results.
- Handle supplies, reagents, and kits with powder-free gloves at all times to avoid contamination and change gloves between removal of used consumables and loading of new consumables.
- Handle specimens carefully with powder-free gloves at all times. Open one tube or specimen at a time to prevent specimen contamination. Change gloves between specimens.
- With PCR tests, there is a possibility of obtaining false positive results due to amplicon-based contamination. Strict adherence to the laboratory's decontamination procedures, following the "Verigene Daily Maintenance" protocol, and careful disposal of consumables into biohazard waste containers after completion of the test are all critical for guarding against false positive results.
- Biological specimens such as respiratory specimens, stool, tissues, body fluids, and blood of humans are potentially infectious. When handling and/or transporting human specimens, follow all applicable regulations mandated by local, state/provincial, and federal agencies for the handling/transport of etiologic agents.

WARNINGS AND PRECAUTIONS – INSTRUMENTS

A. General Instrument Safety

WARNING: Use this product only as specified in this document. Using this instrument in a manner not specified by Nanosphere may result in personal injury or damage to the instrument. Anyone who operates the instrument must have:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Safety Data Sheets (SDS).

B. Electrical Shock Hazard

WARNING: Severe electrical shock can result from operating the instrument without the instrument covers or back panels in place. Do not remove instrument covers or panels. High-voltage contacts are exposed when instrument covers or panels are removed from the instrument. If service is required outside the U.S., contact your local Nanosphere distributor.

WARNINGS AND PRECAUTIONS – REAGENTS AND CONSUMABLES

A. Toxicity of Reagents

- Exposure to chemicals sealed inside the Test Cartridge is hazardous in case of skin contact, respiratory inhalation or ingestion. There is a very small amount of formamide (≤1% v/v). Protective disposable gloves, laboratory coats, and eye protection should be worn when handling specimens, Extraction Trays, Amplification Trays, and Test Cartridges.
- See Safety Data Sheets (SDS) for toxicity information. Safety Data Sheets (SDS) are available at www.nanosphere.us/support.
- An SDS with more information is available for the Test Cartridge, Amplification Tray and Extraction Tray at www.e-labeling.eu and at www.nanosphere.us/support, or upon request from Nanosphere, Inc.

B. Waste Disposal

- The Amplification Tray contains amplification reagents and internal controls. Dispose of the Amplification Tray in accordance with national, state, and local regulations.
- The Extraction Tray contains residual nucleic acids, extraction reagents, and residual sample. It also contains a residual volume of the sample buffer which contains formamide, a teratogen. Dispose of the Extraction Tray in accordance with national, state, and local regulations.
- The Test Cartridge contains residual nucleic acids and hybridization reagents. It also contains a residual volume of the sample buffer which contains formamide, a teratogen. Dispose of the Test Cartridge in accordance with national, state, and local regulations.

EXPECTED VALUES

In the **RP *Flex*** Clinical Evaluation, 2386 prospectively collected fresh and frozen specimens were obtained from six medium- to large-sized healthcare institutions geographically distributed across the United States and one in Mexico. The number and percentage of positive cases (positivity rate) determined by **RP *Flex*** stratified by geographic region and collection site for each of the organisms detected by the test are presented in **Tables 5-7**. Overall, **RP *Flex*** detected at least one target in 40.1% (957/2386) of prospectively collected specimens. In routine practice, detection rates may vary depending on the institution, geographical location, and patient population.

Table 5: Expected Value (As Determined by RP *Flex*) Summary by Collection Site for the RP *Flex* Prospective Clinical Evaluation (Fresh Prospective Specimens) (July 2014 – November 2014)

		Geographic Region/Division						*Total*
Target	*Region*	*Mid-Atlantic*	*Northeast*	*Midwest*		*Southwest*	*Mexico*	
	US State	*MD*	*NY*	*IN*	*MI*	*NM*	*N/A*	
	Site	*1*	*2*	*3*	*4*	*5*	*6*	
	Total n=	34	54	248	4	437	38	815
Influenza A	POS n=	0	0	1	0	8	1	10
	% Pos.	-	-	0.4	-	1.8	2.6	1.2
Influenza A subtype H1	POS n=	0	0	1	0	1	0	2
	% Pos.	-	-	0.4	-	0.2	-	0.2
Influenza A subtype H3	POS n=	0	0	0	0	6	1	7
	% Pos.	-	-	-	-	1.4	2.6	0.9
Influenza B	POS n=	0	1	0	0	8	3	12
	% Pos.	-	1.8	-	-	1.8	7.9	1.5
RSV A	POS n=	0	0	1	0	0	0	1
	% Pos.	-	-	0.4	-	-	-	0.1
RSV B	POS n=	0	0	2	0	0	1	3
	% Pos.	-	-	0.8	-	-	2.6	0.4
Parainfluenza 1	POS n=	0	0	0	0	0	0	0
	% Pos.	-	-	-	-	-	-	-
Parainfluenza 2	POS n=	2	1	2	0	8	0	13
	% Pos.	5.9	1.8	0.8	-	1.8	-	1.6
Parainfluenza 3	POS n=	0	2	1	0	5	0	8
	% Pos.	-	3.6	0.4	-	1.1	-	1.0
Parainfluenza 4	POS n=	0	0	0	0	1	0	1
	% Pos.	-	-	-	-	0.2	-	0.1
Adenovirus	POS n=	0	1	16	0	26	0	43
	% Pos.	-	1.8	6.5	-	5.9	-	5.3
hMPV	POS n=	0	0	2	1	0	1	4
	% Pos.	-	-	0.8	25.0	-	2.6	0.5
Rhinovirus	POS n=	7	16	57	1	145	5	231
	% Pos.	20.6	29.1	30.0	25.0	33.2	13.2	28.3
Bordetella parapertussis/ bronchiseptica	POS n=	1	0	1	0	0	0	2
	% Pos.	5.9	-	0.4	-	-	-	0.2
Bordetella pertussis	POS n=	0	0	1	0	1	0	2
	% Pos.	-	-	0.4	-	0.2	-	0.2
Bordetella holmesii	POS n=	0	1	0	0	0	0	1
	% Pos.	-	1.8	-	-	-	-	0.1

Table 6: Expected Value (As Determined by RP *Flex*) Summary by Collection Site for the RP *Flex* Prospective Clinical Evaluation (Fresh Prospective Specimens) (February 2015 – March 2015)

Target		*Geographic Region/Division*		*Total*
	Region	*Northeast*	*Midwest*	
	US State	*NY*	*WI*	
	Site	*2*	*7*	
	Total n=	147	107	254
Influenza A	POS n=	9	0	9
	% Pos.	6.2	-	3.5
Influenza A subtype H1	POS n=	0	0	0
	% Pos.	-	-	-
Influenza A subtype H3	POS n=	9	0	9
	% Pos.	6.2	-	3.5
Influenza B	POS n=	31	13	44
	% Pos.	21.2	12.1	17.3
RSV A	POS n=	8	4	12
	% Pos.	5.5	3.7	4.7
RSV B	POS n=	6	3	9
	% Pos.	3.8	2.8	3.5
Parainfluenza 1	POS n=	0	0	0
	% Pos.	-	-	-
Parainfluenza 2	POS n=	1	0	1
	% Pos.	0.7	-	0.4
Parainfluenza 3	POS n=	1	4	5
	% Pos.	0.7	3.7	2.0
Parainfluenza 4	POS n=	2	0	2
	% Pos.	1.4	-	0.8
Adenovirus	POS n=	1	3	4
	% Pos.	0.7	2.8	1.6
hMPV	POS n=	7	4	11
	% Pos.	4.8	3.7	4.3
Rhinovirus	POS n=	8	7	15
	% Pos.	5.5	6.5	5.9
Bordetella parapertussis/ bronchiseptica	POS n=	0	0	0
	% Pos.	-	-	-
Bordetella pertussis	POS n=	0	0	0
	% Pos.	-	-	-
Bordetella holmesii	POS n=	0	0	0
	% Pos.	-	-	-

Table 7: Expected Value (As Determined by RP *Flex*) Summary by Collection Site for the RP *Flex* Prospective Clinical Evaluation (Frozen Prospective Specimens) (October 2013 – March 2014)

Target	Midwest- Site 3				
	Total n=1317				
Influenza A	POS n=	52	Parainfluenza 3	POS n=	4
	% Pos.	3.9		% Pos.	0.3
Influenza A subtype H1	POS n=	49	Parainfluenza 4	POS n=	20
	% Pos.	3.7		% Pos.	1.5
Influenza A subtype H3	POS n=	1	Adenovirus	POS n=	68
	% Pos.	0.1		% Pos.	5.2
Influenza B	POS n=	1	hMPV	POS n=	37
	% Pos.	0.1		% Pos.	2.8
RSV A	POS n=	7	Rhinovirus	POS n=	207
	% Pos.	0.5		% Pos.	15.7
RSV B	POS n=	185	*Bordetella parapertussis/ bronchiseptica*	POS n=	1
	% Pos.	14.0		% Pos.	0.1
Parainfluenza 1	POS n=	29	*Bordetella pertussis*	POS n=	8
	% Pos.	2.2		% Pos.	0.6
Parainfluenza 2	POS n=	1	*Bordetella holmesii*	POS n=	0
	% Pos.	0.1		% Pos.	-

PERFORMANCE CHARACTERISTICS

The results of the analytical and clinical studies conducted to establish the performance characteristics of **RP *Flex*** are provided below.

A. Clinical Performance

Clinical studies were conducted at multiple external clinical study sites to evaluate the performance of **RP *Flex*** by comparing viral and bacterial test results to an FDA-cleared molecular respiratory panel and/or PCR amplification followed by confirmatory bi-directional sequencing (PCR/BDS). Subjects included individuals whose routine care called for respiratory pathogen testing.

There were 3299 specimens enrolled in the clinical trials; 1082 of which were prospectively collected fresh specimens, 1330 of which were prospectively collected frozen specimens, 526 of which were selected archived frozen specimens, and 361 of which were contrived frozen specimens.

One hundred and fifty-two (152) specimens resulted in an initial **RP *Flex*** "No Call" for a No Call rate of 4.6% (145/3299 specimens) (95% CI: 3.9% - 5.4%). Seventeen (17) specimens incurred an initial Pre-Analysis Error (PAE), resulting in a PAE rate of 0.5% (17/3299 specimens). The initial total No Call and PAE rate observed during the clinical trials is 5.1% (169/3299 specimens) (95% CI: 4.4% - 5.9%). Of the one hundred and fifty-two (152) initial No Calls, all except fifteen (15) repeated specimens yielded a valid test result upon retesting and of the seventeen (17) initial PAEs, all repeated specimens yielded a valid call upon repeat. The final No Call rate was 0.5% (15/3299 specimens) (95% CI: 0.3% - 0.7%) and the final PAE rate was 0% (0/3299 specimens) for a total final valid test rate of 99.5% (3284/3299 specimens) (95% CI: 99.3% - 99.7%).

Table 8 below provides a summary of demographic information for the 2412 prospectively collected specimens (1082 fresh and 1330 frozen) enrolled in the clinical trials.

Table 8: Summary of Demographic Information for the Prospectively Collected Specimens Enrolled

Age Range	*Prospective Fresh*		*Prospective Frozen*		*Combined*	
	No. of Specimens	*Percentage*	*No. of Specimens*	*Percentage*	*No. of Specimens*	*Percentage*
0-1	151	14.0%	165	12.4%	316	13.1%
>1-5	176	16.3%	382	28.7%	558	23.1%
>5-12	73	6.7%	98	7.4%	171	7.1%
>12-21	74	6.8%	67	5.0%	141	5.8%
>21-65	426	39.4%	275	20.7%	701	29.1%
>65	163	15.1%	155	11.7%	318	13.2%
Not Provided	19	1.8%	188	14.1%	207	8.6%
Total	1082	100%	1330	100%	2412	100%

There were eighteen (18) specimens excluded from the clinical trial due to protocol violations and fifteen (15) specimens which yielded a final No Call result. These specimens were not included in the valid dataset utilized in the performance analyses. Therefore, a total of 3266 specimens were analyzed in this clinical evaluation to establish clinical performance of the test; 1069 of which were prospectively collected fresh specimens, 1317 of which were prospectively collected frozen specimens, 520 of which were selected archived frozen specimens, and 360 of which were contrived frozen specimens.

The clinical performance of **RP *Flex*** is summarized below in Table **9** for Influenza A, Influenza A/H1, Influenza A/H3, Influenza B, RSV A, and RSV B; in Table **10** for Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, and Parainfluenza 4; in **Table 11** for Adenovirus, human Metapneumovirus, and Rhinovirus; and in **Table 12** for *Bordetella pertussis, Bordetella parapertussis/bronchiseptica*, and *Bordetella holmesii.*

Table 9: Results Stratified by Target Analyte – Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza B, Respiratory Syncytial Virus (RSV) A, Respiratory Syncytial Virus (RSV) B

	Specimen Type		*n*=	*% Agreement (95% CI)*	
				Positive	*Negative*
Influenza A	Prospectively Collected	Fresh	1049	100% 12/12 (75.7 - 100)	99.4% 1030/1037[c] (98.6 – 99.7)
		Frozen	1144	97.9% 46/47[a] (88.9 – 99.6)	99.4% 1091/1097[d] (98.8 – 99.7)
		All	2193	98.3% 58/59 (91.0 – 99.7)	99.4% 2121/2134 (99.0 – 99.6)
	Selected		513	99.2% 122/123[b] (95.5 – 99.9)	99.5% 387/390[e] (97.8 – 99.7)
	Contrived		360	-	100% 360/360 (98.9 – 100)
Influenza A subtype H1	Prospectively Collected	Fresh	1048	-	99.8% 1046/1048[h] (99.3 – 99.9)
		Frozen	1144	97.8% 45/46[f] (88.7 – 99.6)	99.6% 1092/1096[i] (99.1 – 99.9)
		All	2190	97.8% 45/46 (88.7 – 99.6)	99.7% 2138/2144 (99.4 – 99.9)
	Selected		512	97.6% 40/41[g] (87.4 – 99.6)	99.6% 469/471[j] (98.5 – 99.9)
	Contrived		360	-	100% 360/360 (98.9 – 100)
Influenza A subtype H3	Prospectively Collected	Fresh	1048	100% 12/12 (75.7 – 100)	99.6% 1032/1036[k] (99.0 – 99.8)
		Frozen	1142	100% 1/1 (20.6 – 100)	100% 1141/1141 (99.7 – 100)
		All	2190	100% 13/13 (77.2 – 100)	99.8% 2173/2177 (99.5 – 99.9)
	Selected		512	100% 82/82 (95.5 – 100)	99.5% 428/430[l] (98.3 – 99.9)
	Contrived		360	-	100% 360/360 (98.9 – 100)
Influenza B	Prospectively Collected	Fresh	1052	98.0% 49/50[m] (89.5 – 99.6)	99.3% 995/1002[n] (98.6 – 99.7)
		Frozen	1145	-	99.9% 1144/1145[o] (99.5 – 100)
		All	2197	98.0% 49/50 (89.5 – 99.6)	99.6% 2139/2147 (99.3 – 99.8)
	Selected		516	100% 26/26 (87.1 – 100)	99.6% 488/490[p] (98.5 – 99.9)
	Contrived		360	-	100% 360/360 (98.9 – 100)
RSV A	Prospectively Collected	Fresh	1049	100% 11/11 (74.1 – 100)	99.8% 1036/1038[r] (99.3 – 99.9)
		Frozen	1121	100% 6/6 (61.0 – 100)	99.9% 1114/1115[s] (99.5 – 100)
		All	2170	100% 17/17 (81.6 – 100)	99.9% 2150/2153 (99.6 – 99.9)
	Selected		498	94.8% 55/58[q] (85.9 – 98.2)	99.3% 437/440[t] (98.0 – 99.8)
	Contrived		360	-	100% 360/360 (98.9 – 100)
RSV B	Prospectively Collected	Fresh	1049	100% 8/8 (67.6 – 100)	99.6% 1037/1041[u] (96.7 – 98.6)
		Frozen	1121	100% 165/165 (97.7 – 100)	97.9% 936/956[v] (96.8 – 98.6)
		All	2170	100% 173/173 (97.8 - 100)	98.8% 1973/1997 (98.2 – 99.2)
	Selected		498	100% 23/23 (85.7 – 100)	98.5% 468/475[w] (97.0 – 99.3)
	Contrived		360	-	99.7% 359/360 (98.4 – 99.9)

[a-b] Influenza A was not detected in 1/1 false negative samples using PCR/BDS analysis.

[c] Influenza A was not detected in 1/7 and detected in 1/7 false positive samples using PCR/BDS analysis. Discordant analysis was not performed in 5/7 false positive samples.
[d] Influenza A was not detected in 2/6 false positive samples using PCR/BDS analysis. Discordant analysis was not performed in 4/6 false positive samples.
[e] Influenza A was not detected in 2/3 false positive samples using PCR/BDS analysis. Discordant analysis was not performed in 1/3 false positive samples.
[f-g] Influenza A/H1 was not detected in 1/1 false negative sample using PCR/BDS analysis.
[h] Influenza A/H1 discordant analysis using PCR/BDS was not performed in 2/2 false positive samples.
[i] Influenza A/H1 was not detected in 2/4 and detected in 1/4 false positive samples using PCR/BDS analysis. Discordant analysis was not performed in 1/4 false positive samples.
[j] Influenza A/H1was not detected in 2/2 false positive samples using PCR/BDS analysis.
[k] Influenza A/H3 was not detected in 3/4 and detected in 1/4 false positive samples using PCR/BDS analysis.
[l] Influenza A/H3 was not detected in 2/2 false positive samples using PCR/BDS analysis.
[m] Influenza B was not detected in 1/1 false negative sample using PCR/BDS analysis.
[n] Influenza B was not detected in 4/7 and detected in 2/7 false positive samples using PCR/BDS analysis. Discordant analysis was not performed in 1/7 false positive samples.
[o] Influenza B was not detected in 1/1 false positive sample using PCR/BDS analysis.
[p] Influenza B was not detected in 2/2 false positive samples using PCR/BDS analysis.
[q] Discordant analysis using PCR/BDS was not performed for the RSV A false negative samples, as PCR/BDS is part of the reference method algorithm for this target.
[r] RSV A was not detected in 1/2 and detected in 1/2 false positive samples using PCR/BDS analysis.
[s] RSV A was not detected in 1/1 false positive sample using PCR/BDS analysis.
[t] RSV A was not detected in 1/3 and detected in 1/3 false positive samples using PCR/BDS analysis. Discordant analysis was not performed in 1/3 false positive samples.
[u] RSV B was detected in 2/4 and was not detected in 2/4 false positive samples using PCR/BDS analysis.
[v] RSV B was not detected in 16/20 and detected in 2/20 false positive samples using PCR/BDS analysis. Discordant analysis was not performed in 2/20 false positive samples.
[w] RSV B was not detected in 5/7 false positive samples using PCR/BDS analysis. Discordant analysis was not performed in 2/7 false positive samples.

Table 10: Results Stratified by Target Analyte – Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4

	Specimen Type		*n*=	*% Agreement (95% CI)*	
				Positive	*Negative*
Parainfluenza 1	Prospectively Collected	Fresh	1052	-	100% 1052/1052 (99.6 – 100)
		Frozen	1145	90.0% 27/30[a] (74.4 – 96.5)	99.8% 1113/1115[b] (99.3 – 99.9)
		All	2197	90.0% 27/30 (74.4 – 96.5)	99.9% 2165/2167 (99.7 – 100)
	Selected		516	100% 50/50 (92.9 – 100)	100% 466/466 (99.2 – 100)
	Contrived		360	-	100% 360/360 (98.9 – 100)
Parainfluenza 2	Prospectively Collected	Fresh	1052	100% 11/11 (74.1 – 100)	99.7% 1038/1041[d] (99.2 – 99.9)
		Frozen	1145	50.0% 1/2[c] (9.5 – 90.5)	100% 1143/1143 (99.7 – 100)
		All	2197	92.3% 12/13 (66.7 – 98.6)	99.9% 2181/2184 (99.6 – 99.9)
	Selected		516	100% 28/28 (87.9 – 100)	99.8% 487/488[e] (98.8 – 100)
	Contrived		360	-	99.7% 359/360 (98.4 – 99.9)
Parainfluenza 3	Prospectively Collected	Fresh	1052	83.3% 10/12[f] (55.2 – 95.3)	99.7% 1037/1040[h] (99.2 – 99.9)
		Frozen	1145	80.0% 4/5[g] (37.5 – 96.4)	100% 1140/1140 (99.7 – 100)
		All	2197	82.4% 14/17 (59.0 – 93.8)	99.9% 2177/2180 (99.6 – 99.9)
	Selected		516	100% 31/31 (89.0 – 100)	100% 485/485 (99.2 – 100)
	Contrived		360	-	100% 360/360 (98.9 – 100)
Parainfluenza 4	Prospectively Collected	Fresh	1052	100% 3/3 (43.8 – 100)	100% 1049/1049 (99.6 – 100)
		Frozen	1145	76.2% 16/21[i] (54.9 – 89.4)	99.6% 1120/1124[j] (99.1 – 99.9)
		All	2197	79.2% 19/24 (59.3 – 90.8)	99.8% 2169/2173 (99.5 – 99.9)
	Selected		516	100% 41/41 (91.4 – 100)	99.6% 473/475[k] (98.5 – 99.9)
	Contrived		360	-	100% 360/360 (98.9 – 100)

[a] Parainfluenza 1 was not detected in 3/3 false negative samples using PCR/BDS analysis.
[b] Parainfluenza 1 was detected in 2/2 false positive samples using PCR/BDS analysis.
[c] Parainfluenza 2 was not detected in 1/1 false negative sample using PCR/BDS analysis.
[d] Parainfluenza 2 was not detected in 2/3 and detected in 1/3 false positive samples using PCR/BDS analysis.
[e] Parainfluenza 2 was not detected in 1/1 false negative sample using PCR/BDS analysis.
[f] Parainfluenza 3 was not detected in 2/2 false negative samples using PCR/BDS analysis.
[g] Parainfluenza 3 was not detected in 1/1 false negative sample using PCR/BDS analysis.
[h] Parainfluenza 3 was not detected in 3/3 false positive samples using PCR/BDS analysis.
[l] Parainfluenza 4 was not detected in 2/5 and detected in 2/5 false negative samples using PCR/BDS analysis. Discordant analysis was not performed in 1/5 false negative samples.
[j] Parainfluenza 4 was not detected in 3/4 and detected in 1/4 false positive samples using PCR/BDS analysis.
[k] Parainfluenza 4 was not detected in 1/2 false positive samples using PCR/BDS analysis. Discordant analysis was not performed in 1/2 false positive samples.

Table 11: Results Stratified by Target Analyte – Adenovirus, Human Metapneumovirus (hMPV), Rhinovirus

	Specimen Type		*n*=	*% Agreement (95% CI)*	
				Positive	*Negative*
Adenovirus	Prospectively Collected	Fresh	1052	91.7% 22/24[a] (74.1 – 97.7)	98.2% 1009/1028[d] (97.1 – 98.8)
		Frozen	1145	81.8% 27/33[b] (65.6 – 91.4)	96.4% 1072/1112[e] (95.1 – 97.3)
		All	2197	86.0% 49/57 (74.7 – 92.7)	97.2% 2081/2140 (96.5 – 97.9)
	Selected		516	97.4% 38/39[c] (86.8 – 99.5)	98.3% 469/477[f] (96.7 – 99.1)
	Contrived		360	-	99.4% 358/360 (98.0 – 99.8)
hMPV	Prospectively Collected	Fresh	1052	100% 10/10 (72.2 – 100)	99.5% 1037/1042[h] (98.9 – 99.8)
		Frozen	1145	100% 36/36 (90.4 - 100)	99.9% 1108/1109[i] (99.5 – 100)
		All	2197	100% 46/46 (92.3 - 100)	99.7% 2145/2151 (99.4 – 99.9)
	Selected		516	92.6% 25/27[g] (76.6 – 97.9)	99.8% 488/489[j] (98.8 – 100)
	Contrived		360	-	99.4% 358/360 (98.0 – 99.8)
Rhinovirus	Prospectively Collected	Fresh	1000	85.9% 214/249[k] (81.1 – 89.7)	95.7% 719/751[n] (94.1 – 97.0)
		Frozen	1122	77.8% 193/248[l] (72.2 – 82.5)	98.3% 859/874[o] (97.2 – 99.0)
		All	2122	81.9% 407/497 (78.3 – 85.0)	97.1% 1578/1625 (96.2 – 97.8)
	Selected		509	80.0% 28/35[m] (64.1 – 90.0)	98.3% 466/474[p] (96.7 – 99.1)
	Contrived		360	-	99.7% 359/360 (98.4 – 99.9)

[a] Adenovirus was detected in 1/2 false negative samples using PCR/BDS analysis. Discordant analysis was not performed in 1/2 false negative samples.
[b] Adenovirus was not detected in 5/6 false negative samples using PCR/BDS analysis. Discordant analysis was not performed in 1/6 false negative samples.
[c] Discordant analysis was not performed in 1/1 false negative Adenovirus sample.
[d] Adenovirus was not detected in 8/19 and detected in 2/19 false positive samples using PCR/BDS analysis. Discordant analysis was not performed in 9/19 false positive samples.
[e] Adenovirus was not detected in 27/40 and detected in 3/40 false positive samples using PCR/BDS analysis. Discordant analysis was not performed in 10/40 false positive samples.
[f] Adenovirus was not detected in 5/8 and detected in 2/8 false positive samples using PCR/BDS analysis. Discordant analysis was not performed in 1/8 false positive samples.
[g] hMPV was not detected in 1/2 false negative samples using PCR/BDS analysis. Discordant analysis was not performed in 1/2 false negative samples.
[h] hMPV was not detected in 3/5 and detected in 2/5 false positive samples using PCR/BDS analysis.
[l] hMPV was detected in 1/1 false positive sample using PCR/BDS analysis.
[j] Discordant analysis was not performed in 1/1 false positive hMPV sample.
[k-m] Discordant analysis using PCR/BDS was not performed for the Rhinovirus false negative samples, as PCR/BDS is part of the comparator method algorithm for this target.
[n] Rhinovirus was not detected in 19/32 and detected in 12/32 false positive samples using PCR/BDS analysis. Discordant analysis was not performed in 1/32 false positive samples.
[o] Rhinovirus was not detected in 11/15 and detected in 2/15 false positive samples using PCR/BDS analysis. Discordant analysis was not performed in 2/15 false positive samples.
[p] Rhinovirus was not detected in 5/8 and detected in 1/8 false positive samples using PCR/BDS analysis. Discordant analysis was not performed in 2/8 false positive samples.

Table 12: Results Stratified by Target Analyte – *Bordetella parapertussis/bronchiseptica, Bordetella pertussis, Bordetella holmesii*

	Specimen Type		*n*=	*% Agreement (95% CI)*	
				Positive	*Negative*
*Bordetella parapertussis/bronchiseptica**	Prospectively Collected	Fresh	1041	100% 2/2 (34.2 – 100)	100% 1039/1039 (99.6 – 100)
		Frozen	1255	-	99.9% 1254/1255[b] (99.5 – 100)
		All	2296	100% 2/2 (34.2 – 100)	99.9% 2290/2291 (99.8 – 100)
	Selected		463	71.4% 5/7[a] (35.9 – 91.8)	99.8% 455/456[c] (98.8 – 100)
	Contrived		360	100% 104/104 (96.4 – 100)	100% 256/256 (98.5 – 100)
Bordetella pertussis	Prospectively Collected	Fresh	1052	100% 1/1 (20.6 – 100)	99.9% 1050/1051[e] (99.5 – 100)
		Frozen	1145	100% 7/7 (64.6 - 100)	99.9% 1137/1138[f] (99.5 – 100)
		All	2197	100% 8/8 (67.6 – 100)	99.9% 2187/2189 (99.7 – 100)
	Selected		516	96.6% 28/29[d] (82.8 – 99.4)	100% 487/487 (99.2 – 100)
	Contrived		360	-	100% 360/360 (98.9 – 100)
*Bordetella holmesii**	Prospectively Collected	Fresh	1043	100% 1/1 (20.6 – 100)	100% 1042/1042 (99.6 – 100)
		Frozen	1263	-	100% 1263/1263 (99.7 – 100)
		All	2306	100% 1/1 (20.6 – 100)	100% 2305/2305 (99.8 – 100)
	Selected		490	50% 1/2[g] (9.4 – 90.1)	100% 488/488 (99.2 – 100)
	Contrived		360	100% 56/56 (93.6 – 100)	100% 304/304 (98.6 – 100)

* PCR/BDS analysis is the comparator method for these targets.

[a] Repeat PCR/BDS was performed. *B. parapertussis/bronchiseptica* was not detected in 1/2 and detected in 1/2 false negative samples. No *B. bronchiseptica* was identified by PCR/BDS in all 7 comparator positive specimens.

[b] Repeat PCR/BDS was performed. *B. parapertussis/bronchiseptica* was not detected in 1/1 false positive sample.

[c] Repeat PCR/BDS was performed. *B. parapertussis/bronchiseptica* was not detected in 1/1 false positive sample.

[d] *Bordetella pertussis* was not detected in 1/1 false negative sample using PCR/BDS analysis.

[e-f] *Bordetella pertussis* was not detected in 1/1 false positive samples using PCR/BDS analysis.

[g] Repeat PCR/BDS was performed. *B. holmesii* was not detected in 1/2 false negative samples. Contamination from a strong positive contrived *B. holmesii* sample in a neighboring well during the original PCR/BDS analysis is highly suspected.

The mixed infections tables for the prospective clinical specimens are provided below. In summary, there were a total of ninety-one (91) mixed infections detected by **RP *Flex*** in prospectively collected specimens (fresh and frozen). The comparator methods identified an additional twenty-three (23) mixed infections in prospectively collected specimens.

Table 13 and **Table 14** list the distinct mixed infection combinations detected in the prospective clinical studies.

Table 13: Distinct Co-infection Combinations Detected by the RP *Flex* in the Prospective Clinical Trial

Distinct Co-infection Combinations Detected by the RP *Flex*				Total Co-infections	Number of Discrepant Co-infections[a]	Discrepant Analyte(s)[a]
Analyte 1	Analyte 2	Analyte 3	Analyte 4			
Adenovirus	Rhinovirus			27	18	Adenovirus (16); Rhinovirus (3)
Rhinovirus	RSV B			22	7	Rhinovirus (4); RSV B (4)
Adenovirus	RSV B			5	1	Adenovirus (1); RSV B (1)
Adenovirus	Rhinovirus	RSV B		3	3	Adenovirus (3); RSV B (1)
Adenovirus	hMPV			3	1	Adenovirus (1)
Rhinovirus	Parainfluenza 1			3	1	Rhinovirus (1)
Rhinovirus	Parainfluenza 4			3	1	Parainfluenza 4 (1)
Rhinovirus	RSV A			3	2	Rhinovirus (2)
Rhinovirus	Parainfluenza 3			2	1	Rhinovirus (1); Parainfluenza 3 (1)
Rhinovirus	hMPV			2	1	hMPV (1)
Adenovirus	Influenza A and A/H1	Influenza B	RSV B	1	1	Adenovirus (1); Influenza A and A/H1 (1); Influenza B (1); RSV B (1)
Adenovirus	Rhinovirus	Parainfluenza 1		1	0	N/A
Adenovirus	Rhinovirus	*B. pertussis*		1	0	N/A
Rhinovirus	Parainfluenza 2			1	1	Rhinovirus (1)
Adenovirus	Parainfluenza 4			1	0	N/A
Adenovirus	RSV A			1	1	Adenovirus (1); RSV A (1)
Influenza A and A/H1	Parainfluenza 3			1	1	Influenza A and A/H1(1)
Influenza A and A/H3	Parainfluenza 2			1	1	Influenza A and A/H1(1); Parainfluenza 2
Influenza B	RSV B			1	1	RSV (1)
Parainfluenza 1	RSV B			1	1	RSV B (1)
Parainfluenza 2	*B. parapertussis/ bronchiseptica*			1	0	N/A
Parainfluenza 3	RSV B			1	0	N/A
Rhinovirus	Influenza A and A/H1			1	1	Rhinovirus (1)
Rhinovirus	Influenza A and A/H3			1	0	N/A
Rhinovirus	Parainfluenza 4	RSV B		1	0	N/A
Adenovirus	Parainfluenza 2			1	1	Parainfluenza 2 (1)
Rhinovirus	*B. pertussis*			1	0	N/A
RSV A	RSV B			1	1	RSV A (1)
Total Co-infections				91		
Total Double Infections				86		
Total Triple Infections				4		
Total Quadruple Infections				1		

[a] A discrepant co-infection or discrepant analyte was defined as one that was detected by RP *Flex* but not detected by the comparator method(s).

Table 14: Additional Distinct Co-infection Combinations Detected by the Comparator Method(s), but not detected by the RP *Flex* in the Prospective Clinical Trial

Distinct Co-infection Combinations Detected by the Comparator Methods				Total Co-infections	Number of Discrepant Co-infections[a]	Discrepant Analyte(s)[a]
Analyte 1	Analyte 2	Analyte 3	Analyte 4			
Rhinovirus	RSV B			9	9	Rhinovirus (9)
Adenovirus	Rhinovirus			3	3	Adenovirus (2); Rhinovirus (3)
Rhinovirus	Parainfluenza 4			3	3	Rhinovirus (2); Parainfluenza 4 (2)
Rhinovirus	Parainfluenza 3			2	2	Parainfluenza 3 (2)
Adenovirus	Rhinovirus	Parainfluenza 4		1	1	Adenovirus (1); Parainfluenza 4 (1)
Adenovirus	Rhinovirus	hMPV		1	1	Rhinovirus (1)
Adenovirus	Parainfluenza 2	RSV B		1	1	Parainfluenza 2 (1)
Rhinovirus	Influenza A and A/H1			1	1	Rhinovirus (1)
Parainfluenza 1	RSV B			1	1	Parainfluenza 1 (1)
Parainfluenza 2	Parainfluenza 4			1	1	Parainfluenza 4 (1)
Total Co-infections				23		
Total Double Infections				20		
Total Triple Infections				3		
Total Quadruple Infections				0		

[a] This table includes only distinct co-infections that were detected by the comparator method(s) but not by RP *Flex*; the remaining co-infections detected by the comparator methods are already represented in Table 12 above.

B. Precision and Reproducibility

The Precision Study of the **RP *Flex*** test involved the testing of a representative test panel daily in duplicate by two (2) operators for twelve (12) non-consecutive days for a total of forty-eight (48) tests per panel sample. The Precision Study used three (3) lots of each of the consumables (cartridges, extraction trays and amplification trays). All precision testing was performed at a single laboratory site with one (1) Verigene reader and twelve (12) Verigene Processor *SP*s.

The test panel, representing all the RP *Flex* analytes except for *B. parapertussis and B. bronchiseptica*, consisted of two (2) negative samples (one negative simulated NPS matrix and one *Staphylococcus aureus* spiked in negative simulated NPS matrix), as well as seven (7) positive mixed samples at two different concentrations for a total of sixteen (16) unique samples. Samples were prepared by spiking previously characterized and quantified organism stocks into simulated NPS matrix at Moderate Positive (5x LoD) and Low Positive (2x LoD) concentrations.

The results of the precision study are summarized in **Table 15,** which provides the percent agreement between the expected results and the obtained results for each sample tested.

The Reproducibility Study of the **RP *Flex*** test evaluated the same test panel as the one used in the Precision Study. The sixteen (16) unique samples were tested in triplicate by two (2) operators over five (5) non-consecutive days at three (3) sites for a total of ninety (90) tests per sample. The results of the Reproducibility Study are summarized in **Table 16.**

Table 15: Precision Study Results

Verigene RP *Flex* Target	Positive Percent Agreement (95% CI)		Negative Percent Agreement* (95% CI)
	Low	Moderate	
Parainfluenza 1	100% 48/48 (92.6-100)	100% 48/48 (92.6-100)	100% 671/671 (99.4-100)
Parainfluenza 2	100% 48/48 (92.6-100)	100% 48/48 (92.6-100)	100% 671/671 (99.4-100)
Parainfluenza 3	100% 48/48 (92.6-100)	95.8 46/48 (86.0-98.8)	100% 671/671 (99.4-100)
Parainfluenza 4	100% 48/48 (92.6-100)	100% 48/48 (92.6-100)	99.9% 670/671 (99.2-100)
RSV A	100% 48/48 (92.6-100)	100% 48/48 (92.6-100)	100% 671/671 (99.4-100)
RSV B	93.8% 45/48 (83.2-97.9)	100% 48/48 (92.6-100)	100% 671/671 (99.4-100)
Influenza A	100% 96/96 (96.2-100)	100% 96/96 (96.2-100)	100% 575/575 (99.3-100)
Influenza A/H1	100% 48/48 (92.6-100)	100% 48/48 (92.6-100)	100% 671/671 (92.4-100)
Influenza A/H3	100% 48/48 (92.6-100)	100% 48/48 (92.6-100)	100% 671/671 (92.4-100)
Influenza B	100% 48/48 (92.6-100)	100% 48/48 (92.6-100)	100% 671/671 (92.4-100)
Rhinovirus	97.9% 47/48 (89.1-99.6)	100% 48/48 (92.6-100)	99.9% 670/671 (99.2-100)
hMPV	100% 48/48 (92.6-100)	100% 48/48 (92.6-100)	100% 671/671 (92.4-100)
Adenovirus	100% 48/48 (92.6-100)	100% 48/48 (92.6-100)	99.7% 669/671 (98.9-99.9)
B. pertussis	100% 48/48 (92.6-100)	97.9% 46/47 (88.9-99.6)	100% 672/672 (99.4-100)
B. holmesii	100% 47/48 (89.1-99.6)	100% 47/47 (92.4-100)	100% 672/672 (99.4-100)

* Negative Percent Agreement (NPA) was determined with all samples that did not contain the target analyte.

Table 16: Reproducibility Study Results

Verigene RP *Flex* Target	Positive Percent Agreement (95% CI)		Negative Percent Agreement* (95% CI)
	Low	Moderate	
Parainfluenza 1	100% 90/90 (96.2-100)	100% 90/90 (96.2-100)	100% 1258/1258 (99.7-100)
Parainfluenza 2	100% 89/89 (95.9-100)	100% 90/90 (96.2-100)	99.8% 1256/1259 (99.3-99.9)
Parainfluenza 3	100% 90/90 (96.2-100)	100% 90/90 (96.2-100)	100% 1258/1258 (99.7-100)
Parainfluenza 4	100% 90/90 (96.2-100)	100% 89/89 (95.9-100)	100% 1259/1259 (99.7-100)
RSV A	98.9% 89/90 (94.0-99.8)	97.8% 88/90 (92.3-99.4)	100% 1258/1258 (99.7-100)
RSV B	100% 90/90 (96.2-100)	100% 90/90 (96.2-100)	99.9% 1257/1258 (99.6-100)
Influenza A	99.4 179/179 (97.9-100)	100% 180/180 (97.9-100)	100% 1079/1079 (99.6-100)
Influenza A/H1	100% 90/90 (96.2-100)	100% 90/90 (96.2-100)	99.8% 1256/1258 (99.4-100)
Influenza A/H3	98.9% 88/89 (93.9-99.8)	100% 90/90 (96.2-100)	99.6% 1254/1259 (99.1-99.8)
Influenza B	100% 90/90 (96.2-100)	100% 90/90 (96.2-100)	99.8% 1255/1258 (99.3-99.9)
Rhinovirus	100% 90/90 (96.2-100)	100% 90/90 (96.2-100)	99.9% 1257/1258 (99.6-100)
hMPV	100% 90/90 (96.2-100)	100% 89/89 (95.9-100)	99.9% 1258/1259 (99.6-100)
Adenovirus	100% 90/90 (96.2-100)	100% 90/90 (96.2-100)	99.8% 1255/1258 (99.3 -99.9)
B. pertussis	96.7% 87/90 (90.7-98.9)	100% 90/90 (96.2-100)	99.9% 1257/1258 (99.6-100)
B. holmesii	100% 90/90 (96.2-100)	100% 90/90 (96.2-100)	99.9% 1257/1258 (99.6-100)

* Negative Percent Agreement (NPA) was determined with all samples that did not contain the target analyte.

C. Analytical Sensitivity (Limit of Detection)

Limit of Detection (LoD) of the **RP *Flex*** test was determined for twenty-eight (28) strains of respiratory pathogens, representing all sixteen (16) **RP *Flex*** reportable target analytes. The LoD was defined as the concentration at which the test produces a positive result ≥95% of the time. Serial dilutions of the strains were tested and the initial tentative LoD confirmed with twenty (20) replicates. To ensure the accuracy of the LoD determination, if the initial detection rate was 100%, a further twenty (20) replicates were performed at the next lower concentration until ≤95% was achieved. The confirmed LoDs for the twenty-eight (28) strains tested and the corresponding LoDs for the **RP *Flex*** test reportable targets are shown in **Table 17** below.

Table 17: Verigene RP *Flex* Limit of Detections

Viral Species and Bacterial Genus	Viral Strains and Bacterial Species	Source	Target	LoD
Adenovirus	C (AdV-1)	Zeptometrix #0810050CF	Adenovirus	1.2×10^{-1} $TCID_{50}$/mL
	B (AdV-3)	Zeptometrix #0810062CF	Adenovirus	1.1×10^{0} $TCID_{50}$/mL
	E (AdV-4)	Zeptometrix #0810070CF	Adenovirus	4.1×10^{-2} $TCID_{50}$/mL
Human Metapneumovirus	Metapneumovirus 9 (A1)	TriCore	hMPV	3.0×10^{1} $TCID_{50}$/mL
	Metapneumovirus 27 (A2)	Zeptometrix #0810164CF	hMPV	1.1×10^{0} $TCID_{50}$/mL
	Metapneumovirus 3 (B1)	Zeptometrix #0810156CF	hMPV	1.0×10^{1} $TCID_{50}$/mL
	Metapneumovirus 8 (B2)	TriCore	hMPV	3.3×10^{0} $TCID_{50}$/mL
Influenza A	Brisbane/59/2007 (H1N1)	TriCore	Influenza A	3.0×10^{1} $TCID_{50}$/mL
			H1	1.0×10^{1} $TCID_{50}$/mL
	California/04/2009pdm09 (H1N1)	TriCore	Influenza A	3.0×10^{1} $TCID_{50}$/mL
			H1	1.0×10^{1} $TCID_{50}$/mL
	Port Chalmers/1/73 (H3N2)	TriCore	Influenza A	3.3×10^{0} $TCID_{50}$/mL
			H3	3.3×10^{0} $TCID_{50}$/mL
	Victoria/361/2011 (H3N2)	Zeptometrix #0810240CF	Influenza A	3.7×10^{-1} $TCID_{50}$/mL
			H3	1.2×10^{-1} $TCID_{50}$/mL

Viral Species and Bacterial Genus	Viral Strains and Bacterial Species	Source	Target	LoD
	Wisconsin/67/05 (H3N2)	Zeptometrix #0810252CF	Influenza A	3.3×10^{0} $TCID_{50}$/mL
			H3	3.3×10^{0} $TCID_{50}$/mL
Influenza B	Brisbane/60/2008	Zeptometrix #0810254CF	Influenza B	1.2×10^{-1} $TCID_{50}$/mL
	Florida/02/2006	TriCore	Influenza B	3.0×10^{1} $TCID_{50}$/mL
	Massachusetts/02/2012	Zeptometrix #0810239CF	Influenza B	1.2×10^{-1} $TCID_{50}$/mL
Parainfluenza	Parainfluenza 1	TriCore (ATCC VR-94)	Parainfluenza 1	9.0×10^{1} $TCID_{50}$/mL
	Parainfluenza 2	TriCore (ATCC VR-92)	Parainfluenza 2	1.0×10^{1} $TCID_{50}$/mL
	Parainfluenza 3	Zeptometrix #0810016CF	Parainfluenza 3	3.3×10^{0} $TCID_{50}$/mL
	Parainfluenza 4a	TriCore (ATCC VR-1378)	Parainfluenza 4	2.7×10^{2} $TCID_{50}$/mL
Rhinovirus	A (Rhinovirus 39)	TriCore (ATCC VR-340)	Rhinovirus	1.0×10^{1} $TCID_{50}$/mL
	B (Rhinovirus 14)	ATCC VR-284	Rhinovirus	9.0×10^{1} $TCID_{50}$/mL
	C (Rhinovirus C41)	UW-Madison	Rhinovirus	2.4×10^{3} PFU/mL
Respiratory Syncytial Virus	RSV A (A2)	TriCore (ATCC VR-1540)	RSV A	3.3×10^{0} $TCID_{50}$/mL
	RSV B (Wash/18537/62)	TriCore (ATCC VR-1580)	RSV B	3.7×10^{-1} $TCID_{50}$/mL
Bordetella	*parapertussis*	ATCC 15311	*B. parapertussis/ bronchiseptica*	2.4×10^{3} CFU/mL
	bronchiseptica	ATCC 786	*B. parapertussis/ bronchiseptica*	2.4×10^{3} CFU/mL
	holmesii	ATCC 51541	*B. holmesii*	2.4×10^{3} CFU/mL
	pertussis	ATCC 9797	*B. pertussis*	8.1×10^{2} CFU/mL

D. Analytical Reactivity (Inclusivity)

The analytical reactivity (inclusivity) of the **RP *Flex*** test was demonstrated with a comprehensive panel of one-hundred and eight (108) strains representing temporal, evolutionary, and geographic diversity for each of the **RP *Flex*** panel organisms. Together with the twenty-eight (28) strains evaluated as part of the Limit of Detection Study, a total of one-hundred and thirty-six (136) strains were evaluated for analytical inclusivity to **RP *Flex*** through empirical testing.

The organisms in the inclusivity panel were prepared in Simulated NPS. Thirteen (13) strains of Influenza A (subtypes H2N2, H2N3, H5N1, H5N3, H7N2, H7N7, H7N9, H9N2 & H10N7) were prepared and tested at a BSL 3 laboratory. Each sample was tested with the **RP *Flex*** in triplicate at an initial concentration 3-fold higher than the LoD determined for each analyte. In cases where the expected targets were not detected in one or more replicates, concentrations at a 3-fold higher level were evaluated.

RP *Flex* demonstrated analytical reactivity to all one-hundred and eight (108) strains tested, with some strains requiring higher titers for detection. The individual strains and concentrations at which positive test results were obtained for all three (3) replicates are presented by target organism in **Table 18** though **Table 26** below.

Table 18: Adenovirus Inclusivity Results

Adenovirus Species	Serotype	Strain #	Source	Concentration ($TCID_{50}$/mL)	Multiples of LoD
A	31	0810073CF	Zeptometrix	1.1×10^{0}	1x
B1	7	VR-7	ATCC	3.3×10^{0}	3x
	21	VR-1099	ATCC	3.3×10^{0}	3x
B2	11	VR-12	ATCC	3.3×10^{0}	3x
	14	0810108CF	Zeptometrix	3.3×10^{0}	3x
	34	VR-716	ATCC	3.3×10^{0}	3x
	35	VR-718	ATCC	1.0×10^{1}	9x
C	2	111010	TriCore	3.3×10^{0}	3x
	5*	0810020CF	Zeptometrix	8.1×10^{2}	729x
	6*	0810111CF	Zeptometrix	2.7×10^{2}	243x
D	26	0810117CF	Zeptometrix	1.1×10^{0}	1x
	37	0810119CF	Zeptometrix	1.1×10^{0}	1x
F	40	0810084CF	Zeptometrix	1.1×10^{0}	1x
	41	0810085CF	Zeptometrix	1.1×10^{0}	1x

* Based on *in silico* analysis, the oligonucleotide identities of all the tested Adenovirus C subtypes have very similar ranges. Based on the investigation of viral stocks titers using a quantitative TaqMan real-time PCR developed at Nanosphere that is specific for all Adenovirus species (note: the primers for the TaqMan assay are not the same primers used in the RP *Flex*), it appears that the amplifiable genome equivalents available in these two adenovirus viral stocks are significantly reduced comparing to that of the other adenovirus stocks tested in the study.

Table 19: Influenza A Inclusivity Results

Influenza A Subtype	Strain	Source	Influenza A		A/H1 or A/H3	
			Concentration ($TCID_{50}$/mL)	Multiple of LoD	Concentration ($TCID_{50}$/mL)	Multiples of LoD
H1N1	A/California/07/2009pdm09	IRR	9.0×10^{1}	3x	9.0×10^{1}	9x
	A/New Caledonia/20/99	Zeptometrix	9.0×10^{1}	3x	9.0×10^{1}	9x
	A/New Jersey/8/76	TriCore	2.7×10^{2}	9x	3.0×10^{1}	3x
	A/NWS/33	TriCore	3.0×10^{1}	1x	3.0×10^{1}	3x
	A/PR/8/ 34	Charles River Labs	3.0×10^{1}	1x	3.0×10^{1}	3x
	A1/Denver/1/57	TriCore	3.0×10^{1}	1x	3.0×10^{1}	3x
	A1/FM/1/47	TriCore	3.0×10^{1}	1x	3.0×10^{1}	3x
	A/ Solomon Islands/3/2006	Zeptometrix	3.0×10^{1}	1x	3.0×10^{1}	3x
	A/Hawaii/15/2001	IRR	2.7×10^{2}	9x	2.7×10^{2}	27x
H3N2	A/ Aichi/ 68	Charles River Labs	1.0×10^{1}	<1x	1.0×10^{1}	3x
	A/ Hong Kong/ 8/ 68	Charles River Labs	3.0×10^{1}	1x	1.0×10^{1}	3x
	A/ Victoria/ 3/ 75*	Charles River Labs	2.4×10^{3}	81x	2.4×10^{3}	729x
	A/Ohio/02/2012	IRR	2.7×10^{2}	9x	2.7×10^{2}	81x
H3N2v	A/Indiana/08/2011	IRR	1.0×10^{1}	<1x	1.0×10^{1}	3x
	A/Minnesota/11/2010**	IRR	2.4×10^{3}	81x	9.0×10^{1}	27x
	A/Indiana/10/2011	IRR	1.0×10^{1}	<1x	3.0×10^{1}	9x
H2N2	Japan/305/1957	MRI	9.0×10^{1}	3x	-	-
H2N3	Mallard/Albert79/03	MRI	9.0×10^{1}	3x	-	-
H5N1	A/Duck/Hunan/795/02	MRI	9.0×10^{1}	3x	-	-
	A/Chicken/Korea/IS/2006	MRI	9.0×10^{1}	3x	-	-
	A/Scaly-breasted Munia/HongKong/2006	MRI	9.0×10^{1}	3x	-	-
H5N3	A/Duck/Singapore/645/1997	MRI	8.1×10^{2}	27x	-	-
H7N2	A/New York/107/2003	MRI	9.0×10^{1}	3x	-	-
H7N7	A/Netherlands/219/2003	MRI	2.7×10^{2}	9x	-	-
	Equine-1/Prague/1956	MRI	9.0×10^{1}	3x	-	-
H7N9	Anhui/01/2013	MRI	9.0×10^{1}	3x	-	-
H9N2	Hong Kong/1073/99	MRI	9.0×10^{1}	3x	-	-
	Chicken/Hong Kong/G9/97	MRI	9.0×10^{1}	3x	-	-
H10N7	Chick/Germany/n/1949	MRI	9.0×10^{1}	3x	-	-

* Based on *in silico* analysis, the oligonucleotide identities of all the tested Influenza A/H3N2 strains have very similar ranges. Based on the investigation of viral stocks titers using a quantitative TaqMan real-time PCR developed at Nanosphere that is specific for Influenza A/H3 strains (note: the primers for the TaqMan assay are not the same primers used in the RP *Flex*), it appears that the amplifiable genome equivalents available in this Influenza A/H3N2 viral stock are significantly reduced comparing to that of the other Influenza A/H3N2 stocks tested in the study.

** Based on *in silico* analysis, the oligonucleotide identities to this strain have slightly lower ranges than the other two H3N2v strains tested.

Table 20: Influenza B Inclusivity Results

Type	Strain	Source	Concentration ($TCID_{50}$/mL)	Multiples of LoD
Influenza B	B/ Allen/45	TriCore	9.0×10^1	3x
	B/Florida/07/2004	TriCore	9.0×10^1	3x
	B/GL/1739/54	TriCore	9.0×10^1	3x
	B/Hong Kong/5/72	ATCC	9.0×10^1	3x
	B/Malaysia/2506/2004	TriCore	9.0×10^1	3x
	B/Maryland/1/59	TriCore	9.0×10^1	3x
	B/Taiwan/2/62	TriCore	9.0×10^1	3x
	B/Wisconsin/01/2010	IRR	9.0×10^1	3x
	B/ Lee/40	Charles River Lab	9.0×10^1	3x
	B/Florida/04/2006	Zeptometrix	9.0×10^1	3x

Table 21: Human Metapneumovirus Inclusivity Results

Subtype	Strain	Source	Concentration ($TCID_{50}$/mL)	Multiples of LoD
hMPV A1	16	Zeptometrix 0810161CF	9.0×10^1	3x
hMPV A2	20	Zeptometrix 0810163CF	9.0×10^1	3x
hMPV B1	5	Zeptometrix 0810158CF	9.0×10^1	3x
hMPV B2	4	Zeptometrix 0810157CF	9.0×10^1	3x
	18	Zeptometrix 0810162CF	9.0×10^1	3x

Table 22: Parainfluenza 1-4 Inclusivity Results

Type		Source/Strain	Concentration ($TCID_{50}$/mL)	Multiples of LoD
Parainfluenza 1		Zeptometrix 0810014CF	2.7×10^2	3x
Parainfluenza 2		Zeptometrix 0810015CF	3.0×10^1	3x
Parainfluenza 3		ATCC VR-93*	2.7×10^2	81x
		BEI NR-3233	3.0×10^1	9x
		TriCore (ATCC VR-1782)	9.0×10^1	27x
Parainfluenza 4	a	Zeptometrix 0810060CF	8.1×10^2	3x
	b	VR-1377	8.1×10^2	3x
		Zeptometrix 0810060BCF	8.1×10^2	3x

* For Parainfluenza 3, the extracted eluate from the three strains tested in the inclusivity study were each evaluated with PCR/bi-directional sequencing, and the sequence information were used to assess the homology to the RP *Flex* oligos. Based on the *in silico* analysis, the three strains have the identical homology to the RP *Flex* oligos, indicating that the apparent difference in sensitivity was not due to sequence diversity in the gene targeted by the RP *Flex*. The apparent variation in the sensitivity of the RP *Flex* test for these strains is likely attributable to inconsistencies in the quantification of the viral stocks.

Table 23: RSV Inclusivity Results

Subtype	Source/Strain	Concentration ($TCID_{50}$/mL)	Multiples of LoD
Respiratory Syncytial Virus A	ATCC VR-26	1.0×10^{1}	3x
	Zeptometrix 0810040ACF	1.0×10^{1}	3x
Respiratory Syncytial Virus B	Zeptometrix 0810040CF	1.1×10^{0}	3x
	ATCC VR-1400	1.1×10^{0}	3x
	ATCC VR-955	3.3×10^{0}	9x

Table 24: Rhinovirus A and B Inclusivity Results

Rhinovirus Species	Strain	Source	Concentration ($TCID_{50}$/mL)	Multiples of LoD
Rhinovirus A	1	Zeptometrix 0810012CFN	2.7×10^{2}	3x
	2	ATCC VR-482	2.7×10^{2}	3x
	7	ATCC VR-1601	2.7×10^{2}	3x
	16	ATCC VR-283	2.7×10^{2}	3x
	34	ATCC VR-507	2.7×10^{2}	3x
	57	ATCC VR-1600	2.7×10^{2}	3x
	77	ATCC VR-1187	2.7×10^{2}	3x
	85	ATCC VR-1195	2.7×10^{2}	3x
Rhinovirus B	3	ATCC VR-483	2.7×10^{2}	3x
	17	ATCC VR-1663	2.7×10^{2}	3x
	27	ATCC VR-1137	2.7×10^{2}	3x
	42	ATCC VR-338	2.7×10^{2}	3x
	83	ATCC VR-1193	2.7×10^{2}	3x

Table 25: Rhinovirus C Inclusivity Results

Rhinovirus Species	Strain	Source	Concentration (PFU/mL)*	Multiples of LoD
Rhinovirus C	C2	UW-Madison	7.3×10^{3}	3x
	C15	UW-Madison	7.3×10^{3}	3x

* As there is no susceptible cell line to grow Rhinovirus C, the strains were cloned into a plasmid vector and transfected into WisL cells (primary human lung fibroblasts). All were sequenced to confirm identity. The titers were established by qPCR using serial dilutions of Rhinovirus 16 as a surrogate to provide actual PFU/mL values for the standard curve. Therefore, it has been assumed that Rhinovirus 16 has similar virulence rates to Rhinovirus C.

Table 26: *Bordetella* Species Inclusivity Results

Bordetella Species	Source	RP Flex Target	Concentration (CFU/mL)	Multiples of LoD
B. pertussis	ATCC 51445	*B. pertussis*	2.4×10^3	3x
	ATCC 10380		2.4×10^3	3x
	ATCC 9340		2.4×10^3	3x
	ATCC BAA-589		2.4×10^3	3x
	ATCC BAA-1335		2.4×10^3	3x
	ATCC 53894		2.4×10^3	3x
	ATCC 9306		2.4×10^3	3x
	ATCC 8467		7.3×10^3	9x
B. parapertussis	ATCC 15237	*Bordetella parapertussis/ bronchiseptica*	7.3×10^3	3x
	ATCC 9305		7.3×10^3	3x
	ATCC BAA-587		7.3×10^3	3x
	ATCC 15989		7.3×10^3	3x
	Zeptometrix 0801461		2.2×10^4	9x
B. bronchiseptica	ATCC 4617	*Bordetella parapertussis/ bronchiseptica*	7.3×10^3	3x
	ATCC 7773		7.3×10^3	3x
	ATCC 785		7.3×10^3	3x
	ATCC 14064		7.3×10^3	3x
	ATCC 10580		7.3×10^3	3x
	ATCC 19395		7.3×10^3	3x
B. holmesii	Zeptometrix 0801464	*B. holmesii*	2.2×10^4	9x
	ATCC 700053		2.4×10^3	1x
	ATCC 700052		2.4×10^3	1x

E. Analytical Specificity (Exclusivity)

One-hundred and seven (107) organisms, consisting of forty-six (46) bacterial/fungal strains (**Table 27**) (tested at 1.0×10^6 CFU/mL), twenty-six (26) viruses (
Table 28), twenty-two (22) in-panel tested in the LoD study, and thirteen (13) additional influenza A virus strains with other hemagglutinin (HA) types (**Table 29**) were tested with **RP *Flex*** to determine analytical specificity (exclusivity).

The viral and bacterial/fungal samples were contrived in Simulated NPS at high concentrations (1.00×10^5 $TCID_{50}$/mL for viral targets and at 1.0×10^6 CFU/mL for bacterial and fungal targets, except for Mumps virus which was tested at the highest available concentration of 1.6×10^4 $TCID_{50}$/mL). Four (4) organisms which were not available as titered stocks were evaluated using genomic DNA at 1.0×10^6 copies/mL. All samples were tested in triplicate with the RP *Flex*.

Table 27: Bacterial and Fungal Organisms Tested for RP *Flex* Analytical Specificity

Genus	Species	Strain Number
Acinetobacter	*baumannii*	ATCC 19606
Bordetella	*avium*	ATCC 35086
Bordetella	*hinzii*	ATCC 51784
Bordetella	*petrii*	ATCC BAA-461
Bordetella	*trematum*	ATCC 700309
Candida	*albicans*	ATCC 18804
Candida	*glabrata*	ATCC 38326
Chlamydophila	*pneumoniae*	ATCC VR-1360
Chlamydia	*trachomatis* Serovar D	ATCC VR-885
Corynebacterium	*pseudodiphtheriticum*	ATCC 10700
Corynebacterium	*diphtheriae*	ATCC 14779
Corynebacterium	*striatum*	ATCC BAA-1293
Escherichia	*coli*	ATCC 25922
Haemophilus	*influenzae*	ATCC 49144
Haemophilus	*parainfluenzae*	ATCC 9796
Klebsiella	*pneumoniae* subsp. *pneumoniae*	ATCC 13883
Lactobacillus	*acidophilus*	Zeptometrix 0801540
Lactobacillus	*plantarum*	ATCC BAA-793
Legionella	*pneumophilia*	ATCC 33152
Legionella	*longbechiae*	ATCC 33462
Legionella	*micdadei*	ATCC 33204
Listeria	*innocua*	ATCC 51742
Listeria	*monocytogenes* serotype 4b	ATCC 19115
Moraxella (Branhamella)	*catarrhalis*	ATCC 43617
Mycobacterium	*tuberculosis*	ATCC BAA-2237D-2[a]
Mycoplasma	*genitalium*	ATCC 49123[a]
Mycoplasma	*hominis*	ATCC 27545-TTR
Mycoplasma	*pneumoniae*	ATCC 15531-TTR
Neisseria	*elongata* subsp. *elongata*	ATCC 25295
Neisseria	*gonorrhoeae*	ATCC 31426
Neisseria	*meningitidis*	ATCC 53415D-5[a]
Neisseria	*lactamica*	ATCC 23970
Neisseria	*mucosa*	ATCC 49233
Neisseria	*sicca*	ATCC 29256
Pneumocystis	*jirovecii*	Erasme-Belgium-Clinical Sample*
Proteus	*vulgaris*	ATCC 6380
Pseudomonas	*aeruginosa*	ATCC 27853
Serratia	*marcescens*	ATCC 29021
Staphylococcus	*aureus* subsp. *aureus*	ATCC 12600
Staphylococcus	*epidermidis*	ATCC 12228
Staphylococcus	*haemolyticus*	ATCC 29970
Streptococcus	*agalactiae*	ATCC 12386
Streptococcus	*pneumoniae*	ATCC 6303
Streptococcus	*pyogenes*	ATCC 14289
Streptococcus	*salivarius*	ATCC 13419
Ureaplasma	*urealyticum*	ATCC 27618[a]

[a] Genomic DNA tested at 1.00×10^6 copies/mL

Table 28: Viral Organisms Tested for RP *Flex* Analytical Specificity

Virus Name	Type	Source/Strain Number
Bocavirus	-	Clinical Sample
Coronavirus	229E	Zeptometrix 0810229CF
Coronavirus	NL63	Zeptometrix 0810228CF
Coronavirus	OC43	Zeptometrix 0810024CF
Coronavirus	HKU1	LIJ-Clinical Sample
Cytomegalovirus	-	ATCC VR-977
Enterovirus A	Type 71	Zeptometrix 0810047CF
Enterovirus A	Coxsackievirus A2	ATCC VR-1550
Enterovirus A	Coxsackievirus A10	Zeptometrix 0810106CF
Enterovirus B	Coxsackievirus A9	Zeptometrix 0810017CF
Enterovirus B	Coxsackievirus B4	ATCC VR-184
Enterovirus B	Coxsackievirus B5	ATCC VR-185
Enterovirus B	Echovirus 6	Zeptometrix 0810076CF
Enterovirus B	Echovirus 9	Zeptometrix 0810077CF
Enterovirus B	Echovirus 11	Zeptometrix 0810023CF
Enterovirus B	Echovirus 30	Zeptometrix 0810078CF
Enterovirus C	Coxsackievirus A21	Zeptometrix 0810235CF
Enterovirus C	Coxsackievirus A24*	ATCC VR-1662
Enterovirus C	Poliovirus 2 (attenuated)*	ATCC VR-301
Enterovirus C	Poliovirus 3 (attenuated)*	ATCC VR-193
Enterovirus D	Type 68*	ATCC VR-561
Epstein Barr Virus	-	Zeptometrix 0810008CF
Herpes Simplex virus	Type 1	Zeptometrix 0810005CF
Measles	-	ATCC VR-24
Mumps virus	-	ATCC VR-106
Varicella-Zoster virus	-	Zeptometrix 0810026CF

Table 29: In-Panel RP *Flex* Organisms (Viruses and Bacteria) and Additional Influenza A Virus Strains with Other Hemagglutinin (HA) Types Tested for Analytical Specificity

Bacteria/Virus Name	Type	Source/Strain Number
Adenovirus A	Type 31	Zeptometrix ×810073CF
Adenovirus D	Type 26	Zeptometrix 0810117CF
Adenovirus D	Type 37	Zeptometrix 0810119CF
Adenovirus F	Type 40	Zeptometrix 0810084CF
Adenovirus F	Type 41	Zeptometrix 0810085CF
Adenovirus E	Type 4	Zeptometrix 0810070CF
Bordetella holmesii	-	ATCC 51541
Bordetella pertussis	-	ATCC 9797
Influenza A /Brisbane/59/2007	H1N1	TriCore
Influenza A /Wisconsin/67/05	H3N2	Zeptometrix N/A
Influenza A/California/04/2009pdm09	H1N1 - pandemic	TriCore
Influenza A/Victoria/361/2011	H3N2	Zeptometrix 0810240CF
Influenza A	H2N2[a]	Japan/305/1957
Influenza A	H5N1[a]	A/Duck/Hunan/795/02
Influenza A	H5N1[a]	A/Chicken/Korea/IS/2006
Influenza A	H5N1[a]	Scaly-breasted Munia/HongKong/2006
Influenza A	H7N2[a]	New York/107/2003
Influenza A	H7N7[a]	Netherlands/219/2003
Influenza A	H7N9[a]	Anhui/01/2013
Influenza A	H9N2[a]	Hong Kong/1073/99
Influenza A	H2N3[a]	Mallard/Albert79/03
Influenza A	H5N3[a]	Duck/Singapore/645/1997
Influenza A	H7N7[a]	Equine-1/Prague/1956
Influenza A	H9N2[a]	Chicken/Hong Kong/G9/97
Influenza A	H10N7[a]	Chick/Germany/n/1949
Influenza B /Florida/02/2006	-	TriCore
Metapneumovirus 9	Type A1	TriCore
Metapneumovirus 8	Type B2	TriCore
Parainfluenza 1	-	TriCore VR-94
Parainfluenza 2	-	TriCore VR-92
Parainfluenza 3	-	Zeptometrix 0810016CF
Parainfluenza 4a	-	TriCore VR-1378
Respiratory Syncytial Virus	Type A2	TriCore VR-1540
Respiratory Syncytial Virus	Type B	TriCore VR-1580
Rhinovirus 14	Type B	TriCore

[a] Prepared and tested at a BSL 3 laboratory.

All of the organisms tested yielded the expected "Not Detected" results at the concentrations tested with the exception of the enteroviruses marked with an asterisk (*) in **Table 28** above, and *Pneumocystis jirovecii* (from a clinical sample) marked with an asterisk in **Table 27** above, which gave "Rhinovirus detected" results in some of the replicates.

Based on *in silico* analyses, a number of Enterovirus strains have a relatively high homology to RP *Flex* Rhinovirus oligos, with some percent identities to Rhinovirus RP *Flex* oligos of 84%. As a result, some cross-reactivity at high titer was expected.

In silico analysis also determined that *Pneumocystis jirovecii* sequences have a maximum Oligo Identity to RP *Flex* targets of 67% and therefore *Pneumocystis jirovecii* was not predicted to be cross-reactive to RP *Flex* Rhinovirus probes. Extracted nucleic acids from all Rhinovirus positive tests of the *Pneumocystis jirovecii* positive clinical sample were evaluated with an analytically validated PCR/BDS Rhinovirus assay. PCR/BDS test results confirmed the presence of Rhinovirus in all samples, indicating that the *Pneumocystis jirovecii* positive clinical sample also contains Rhinovirus nucleic acids.

F. Interference

Microbial Interference

Three (3) representative target organisms detected by **RP *Flex***, Adenovirus 3 (B), Influenza A (H1N1), and *Bordetella pertussis* were evaluated at 3x their respective LoD for potential interference in the presence of seven (7) potentially interfering microorganisms not detected by **RP *Flex***: *Staphylococcus aureus, Neisseria meningitidis, Corynebacterium diphtheria, Haemophilus influenza, Streptococcus pneumoniae, Mycoplasma pneumoniae,* and cytomegalovirus. These seven (7) microorganisms represent the most prevalent microorganisms known to be present in the human upper respiratory tract and therefore the most likely to be encountered in NPS specimens. These normal flora organisms were tested at a concentration of 1.00×10^6 CFU/mL with the exception of *Mycoplasma pneumoniae,* which was tested at 1.00×10^6 CCU/mL, and *Neisseria meningitidis,* which was tested at 1.00×10^6 genomic copies/mL and cytomegalovirus, which was tested at 1.00×10^5 PFU/mL. No interference was observed with the **RP *Flex*** test for any of these samples tested.

Exogenous and Endogenous Substances

A comprehensive interfering substances study was performed to assess the potential effects of endogenous and exogenous substances that can commonly be found in clinical upper respiratory specimens. Three (3) representative target organisms detected by **RP *Flex***, Adenovirus 3 (B), Influenza A (H1N1), and *Bordetella pertussis* were evaluated at 3x their respective LoD for potential interference in the presence of thirty-six (36) potentially interfering exogenous substances (

Table 30). Two (2) endogenous substances were also included, human blood and human DNA. None of the substances at the concentrations tested showed any inhibitory effect on the detection of target respiratory pathogens using the **RP *Flex*** test.

Table 30: Exogenous Substances Evaluated for Interference

Interfering Substance Tested		
Wal-Four® Nasal Spray	Fluticasone furoate	BD Universal Viral Transport Media
Anefrin Nasal Spray	Menthol	Remel M4®
Saline Nasal Spray	Mupirocin	Remel M4-RT®
Similasan Sinus Relief	Tobramycin	Remel M5®
Anbesol® (Anesthetic)	Mucin, bovine submaxillary Type I-S	Remel M6™
Beclomethasone dipropionate	Mucin, porcine stomach Type II	BD Liquid Amies
Dexamethasone	Mucin, porcine stomach Type III	Remel Regan Lowe Semi-Solid Transport Media
Flunisolide	Oseltamivir Phosphate	Ethyl Alcohol, Absolute 200 Proof
Triamcinolone acetonide	Boiron® (Sulfur)	Acetonitrile
Budesonide	Boiron® (Galphimia Glauca)	Copan CLASSIQSwabs (Aluminum applicator, rayon tipped, sterile)
Mometasone furoate	Boiron® (Histaminum Hydrochloricum)	Copan FLOQSwabs (Nylon® regular, sterile)
Fluticasone propinoate	Zanamivir	FluMist® Influenza Vaccine Live, Intranasal

Additionally, all potential interferents were tested in triplicate without **RP *Flex*** target organisms as negative controls. No false positive was observed for the negative controls, except for the MedImmune FluMist® Influenza Vaccine Live, Intranasal Spray (2011-2012 Formula).

G. Carryover/Cross-Contamination Study

The potential for carryover and cross-contamination on the Verigene system was assessed by alternately testing three (3) representative high positive respiratory pathogen samples; Adenovirus 3 (B), Influenza A (H1N1) (both at 1.00×10^5 $TCID_{50}$/mL), and *Bordetella pertussis* (at 1.00×10^6 CFU/mL), followed by testing a negative NPS sample. The high-titer sample was alternated with the negative sample five (5) times on six (6) unique Processor *SPs*. No carryover or cross-contamination was observed.

H. Competitive Interference

In order to assess potential competitive inhibition for **RP *Flex***, binary combinations of all test panel organisms representing all possible dual infections, were evaluated. Contrived samples were prepared in negative simulated NPS matrix, with one panel organism present at a Low Positive titer (3x LoD) and a second organism present at a High Positive titer (1.00×10^5 $TCID_{50}$/mL for viruses, 1.00×10^6 CFU/mL for bacteria). The performance of **Verigene RP *Flex*** was evaluated with each of the one-hundred and eighty-two (182) unique sample combinations tested in replicates of three (3). The results of the Competitive Interference testing are summarized in **Table 31**. No evidence of competitive inhibition was observed at the titers tested.

Table 31: Competitive Interference Testing

		Low Positive Titer Strains (3x LoD)													
Binary Combinations of Strains		Influenza A H1	Influenza A H3	Influenza B	hMPV	RSV A	RSV B	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Parainfluenza 4	Adenovirus	Rhinovirus	*Bordetella pertussis*	*Bordetella holmesii*
High Positive Titer Strains (1.00×10^5 $TCID_{50}$/mL-viral, 1.00×10^6 CFU/mL- bacterial)	Influenza A H1	–	3/3	8/9*	3/3	3/3	3/3	3/3	3/3	3/3	3/3	8/9*	3/3	3/3	8/9*
	Influenza A H3	3/3	–	3/3	3/3	3/3	8/9*	3/3	3/3	3/3	3/3	3/3	8/9*	3/3	3/3
	Influenza B	3/3	3/3	–	3/3	3/3	3/3	3/3	3/3	3/3	3/3	8/9*	3/3	3/3	3/3
	hMPV	3/3	3/3	3/3	–	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	8/9*
	RSV A	3/3	3/3	3/3	3/3	–	8/9*	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	RSV B	3/3	3/3	3/3	3/3	3/3	–	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	Parainfluenza 1	3/3	3/3	3/3	3/3	3/3	3/3	–	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	Parainfluenza 2	3/3	3/3	3/3	3/3	8/9*	3/3	3/3	–	3/3	3/3	3/3	3/3	3/3	3/3
	Parainfluenza 3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	–	3/3	3/3	3/3	3/3	3/3
	Parainfluenza 4	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	–	3/3	3/3	3/3	3/3
	Adenovirus	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	–	3/3	3/3	3/3
	Rhinovirus	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	–	3/3	8/9*
	Bordetella pertussis	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	–	3/3
	Bordetella holmesii	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	–
Total Detection Rate of Low Titer Strain	*No.*	39/39	39/39	44/45	39/39	44/45	49/51	39/39	39/39	39/39	39/39	49/51	44/45	39/39	54/57
	%	100%	100%	98%	100%	98%	96%	100%	100%	100%	100%	96%	98%	100%	95%

* 3/3 indicates that all expected targets for the low positive titer organisms (as well as the high titer organisms) were detected in all three replicates. However, in 10 cases the low titer organism was detected in 2 of the 3 replicates. For each of these 10 cases, an additional 6 tests were performed successfully to yield a final total of 8/9 successful tests. The single discordant result in each of these 10 combinations is likely reflective of the probability for missed detection in samples at the low concentration.

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REF	Catalog number
	Use by YYYY-MM-DD
LOT	Batch code
SN	Serial number
	Manufacturer
	Upper Limit – Temperature limitation
	Upper and Lower Limit – Temperature limitation
	Consult instructions for use
	Harmful
	Flammable
	Irritant
	Toxic

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